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MICROBIAL METAL EXTRACTION AND RECOVERY:

Business Opportunities, Technology Assessments, and Market Forecasts to 1990

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1.0 EXECUTIVE SUMMARY

1.1 PROGRAM OBJECTIVES AND METHODOLOGY

The purpose of this study is to project future market size and characteristics, describe new business opportunities, and critically review the technical status of microbial beneficiation and metal extraction of ores, ore wastes, slags, slimes, etc., and microbial metal concentration from solution.

The information used in conducting this study was obtained by:

(1) A thorough literature and patent review, including extensive use of the authors own holdings and a computer search of:

a. Water Resources Abstracts
b. Biosis Previews
c. Metadex
d. Life Sciences Collection
e. Telegen
f. CA Search
g. Compendex
h. World Aluminum Abstracts
i. Claims/U.S. Patent Abstracts

(2) Discussions with current investigators and practitioners in the field.

(3) Use of available metal demand models, coupled with Gorham International's market forecasts to project the eventual impact of microbial metal extraction and recovery on conventional processes.

1.2 SYNOPSIS OF THE STUDY

The study reviews the history of microbial leaching and metal concentration and the fundamental principles involved in these processes. It reviews the types of ores and other materials which microbes can leach and the metals they can concentrate from solution, the physiology and biochemistry pertinent to bioleaching, and the parameters of the microbial activity which are important and need to be optimized for practical application. The study reviews the principles of genetic engineering in a non-technical way and indicates how genetic engineering might be applied to improve microbes active in leaching and metal concentration. It, furthermore, points out the limitations of this technology as applied to microbes involved in leaching.

The study summarizes new scientific developments in bioleaching and bioconcentration of metals. It reviews the practice of leaching of copper sulfide ore-concentrates and low-grade ores in terms of unit processes. It presents a mathematical method for scale-up of bioleaching of low-grade metal-sulfide ore in dumps, heaps, and in place, and for leaching of metal-sulfide ore concentrate.

The study indicates who is presently recovering metals microbiologically and who is actively conducting R&D activities in this field. It assesses the demand for metals in the next 10 to 20 years and what proportion of that demand may be satisfied by microbial processes. It assesses the need and demand for genetically engineered microbes. It suggests what geographical growth characteristics of microbial leaching and metal recovery from solution would be in the near future and what its likely impact on energy consumption, metals prices and trade patterns might be.

Finally, the study offers general statements on new business opportunities.

1.3 MAJOR FINDINGS AND CONCLUSIONS

1.3.1 Market Characteristics

1. Microbial metal extraction and recovery technologies are expected to have a substantial impact on metal markets in the 1990's. Table E1 below lists forecast quantities and sales values of the metals most likely to be obtained by microbial means in 1990, together with forecast sales of genetically-engineered organisms.

2. To date, a total of 21 metals have been demonstrated to be obtainable by microbial means. The retail sales value in 1990 of

TABLE E1: TOTAL U.S. AND WORLD METAL REQUIREMENTS IN 1990 AND ESTIMATE OF QUANTITY OBTAINED BY MICROBIAL MEANS OF 8 LEADING METALS(a)

			Estimate of Quantity Produced In 1990 By Microbial Means			Likely Microbial Technology To Be Employed(b)			Market Size for Genetically-Engineered Microbes
Metal	Market(d)	Requirement 1990	Quantity	Unit Value(c)	Total Value ($ Million)	B	E	C	($ Million)
Ag	U.S.	225,000,000 tr oz	45,000,000 tr oz	$12 per tr oz	$ 540	X			$ 5
	World	665,000,000 tr oz	133,000,000 tr oz	12 per tr oz	1596	X			16
Al	U.S.	11,400,000 tons	1,710,000 tons	1600 per ton	2736	X	X		27
	World	38,000,000 tons	5,700,000 tons	1600 per ton	9120	X	X		91
Au	U.S.	6,100,000 tr oz	1,525,000 tr oz	425 per tr oz	648	X			6
	World	62,400,000 tr oz	15,600,000 tr oz	425 per tr oz	6630	X			67
Co	U.S.	30,000,000 lbs	4,500,000 lbs	12.50 per lb	56		X	X	1
	World	79,100,000 lbs	11,865,000 lbs	12.50 per lb	148		X	X	2
Cu	U.S.	3,400,000 tonnes	1,700,000 tonnes	1760 per tonne	2292		X	X	23
	World	16,000,000 tonnes	8,000,000 tonnes	1760 per tonne	14080		X	X	141
Ni	U.S.	400,000 tons	100,000 tons	6500 per ton	650		X	X	7
	World	1,620,000 tons	405,000 tons	6500 per ton	2633		X	X	26
Ti	U.S.	804,000 tons	120,600 tons	30000 per ton	3618	X	X		36
	World	2,900,000 tons	435,000 tons	30000 per ton	13050	X	X	-	131
U	U.S.	20,100,000 lbs	10,000,000 lbs	23.50 per lb	235		X	X	2
	World	80,000,000 lbs	40,000,000 lbs	23.50 per lb	940		X	X	9

Total Metal Value Obtained By Microbial Means, 1990, U.S. = $10,775 Million
Total Metal Value Obtained By Microbial Means, 1990, World = $48,197 Million

Total Market Size for Genetically-Engineered Microbes, 1990, U.S. = 107
Total Market Size for Genetically-Engineered Microbes, 1990, World= 483

NOTES: (a) for full table and methodology, see Table 18
(b) B = beneficiation; E = extraction; C = concentration
(c) in 1983 U.S. dollars.
(d) World includes USA and Centrally Planned Economies.

these metals is projected to be $17,786 million (U.S.) and $93,430 million (world), based on 1983 metal prices. The current value of microbially extracted copper and uranium is about $350 million in the U.S., and $1,750 million worldwide. This forecast is strictly speculative, partly because of the risk and uncertainty involved in bringing this technology to fruition, and partly because of the conservative stance of many mining and metal processing firms to developing and utilizing this technology.

3. At the present time, virtually all major copper mining companies practice microbial metal extraction. Approximately 10-20 percent of world copper production is won microbially.

4. Microbial leaching of uranium-containing ores is currently practiced at one mine in Canada. In the 1970's (when demand for uranium was higher) three other Canadian mines used bioleaching to recover uranium.

5. Bioleaching looks particularly viable for geographically remote ore bodies that are far removed from ore processing plants, as is often the case in undeveloped nations. In highly developed nations, installing bioleaching facilities is likely to be cheaper than upgrading existing smelters to meet pollution standards.

6. It is difficult to project the ultimate size of business for genetically engineered microbes in 1990 for metal extraction and concentration, because this technology is too immature to permit accurate forecasting. The value and quality of innocula needed depend on the metal and the source, the pore volume/surface area, loss of potency of the culture, rate of poisoning, etc. as well as any licensing and commercial agreements negotiated between the microbe developer and the metal producer. As an order of magnitude estimate, a engineered-microbe market of $175 million (U.S.) and $900 million (world) in 1990 is considered achievable.

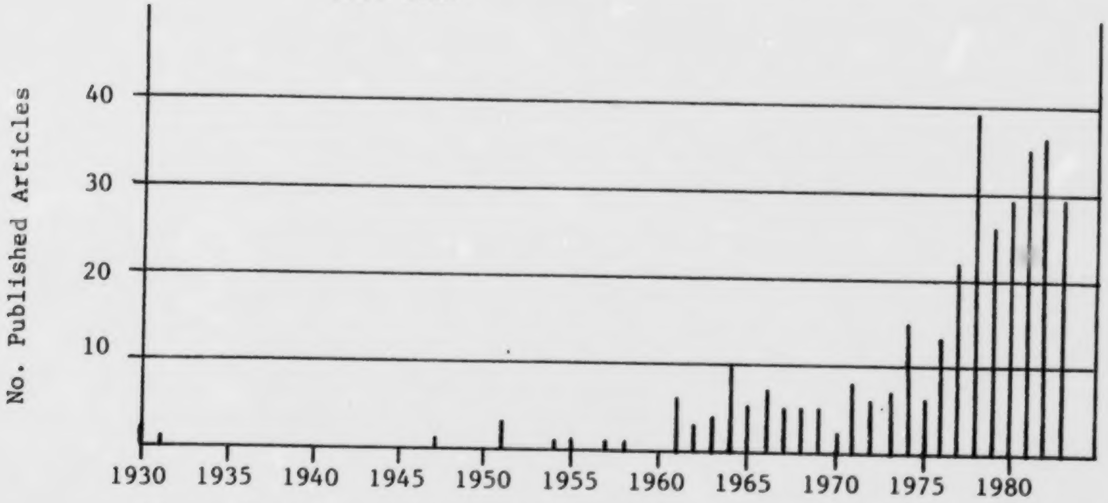
7. The market in 1990 for special equipment and simple inorganic and organic nutrients is likewise difficult to estimate, but is expected to be at least as large as the market for microbes ($175 million - U.S., $900 million - world).

8. Comparing hydrometallurgical, pyrometallurgical, and bioleaching processes, bioleaching is the most energy efficient even for plant sizes one-quarter the size of hydrometallurgical/pyrometallurgical plants.

1.3.2 **General Assessment Conclusions**

1. Microbial metal extraction/concentration biotechnology is still in the infancy stage. Only about 20 patents have appeared on this subject since the first was issued in 1958. The rate of appearance of professional literature articles is shown in Figure E1, below.

Figure E1: Rate of Appearance of Professional Articles and Books on Microbial Metal Extraction/Recovery, 1930-1983*



*Approximate, as publication frequency is based only on those articles cited in this report. Data for 1983 is for the first 6 months of the year only.

2. Except for heap, dump, and in-place bioleaching, the other processes described in this report need further R&D work before they can be fully exploited on a commercial scale. Full realization of economic processes is likely to require 5 to 10 years, depending on the stage in R&D at which a start is made.

3. Microbe/metal interactions can be classified as follows:

(1) metal oxidation
(2) metal reduction
(3) metal chelation
(4) metal methylation
(5) metal sorption
(6) metal transport
(7) metal inhibition

Which interaction(s) is operable depends on the specific metal and organism involved.

4. Microbes can aid in ore beneficiation, metal extraction, and metal recovery from solution. Microbes, however, are not capable of reducing metallic compounds to the metallic state. (The only exception to this is microbial reduction of oxidized mercury, selenium, and tellurium to the elemental form).

5. Up to now, commercial large scale microbial leaching has been practiced only on low-grade copper and uranium ores. The use of microbes to leach, concentrate, or beneficiate many other ores and metals has been successfully demonstrated on a laboratory or pilot scale, however.

6. Increased commercial exploitation of microbial metal recovery will occur if

(1) the rates of extraction/concentration reactions can be increased

(2) accelerated cell multiplication rates can be achieved
(3) interfering biochemical reactions can be eliminated
(4) increased tolerance for toxic metals can be achieved.

Improvement in each of these traits may be achieved by appropriate genetic manipulation.

7. Fears that genetically-engineered microbes used in microbial metal extraction/concentration processes could cause damage to the environment and human health are largely unfounded. Engineered strains are most likely to be less competitive than wild type strains, and, in any case, will find widest application in closed reactors where introduction into the general environment can be easily controlled and avoided.

1.3.3 Technology of Microbial Metal Extraction

1. Within the foreseeable future, biohydrometallurgical extaction could account for 5-50 percent of total metal recoveries.

2. From an economic standpoint, microbial metal extraction is attractive if

(1) the metal value of the ore is too low for conventional extraction;
(2) the metal extraction cannot be easily achieved by purely chemical/physical methods;
(3) the non-biological method of metal extraction is to costly;
(4) the non-biological method produces air/water pollutants whose disposal into the environment is too costly to control.

3. Unlike smelting, biohydrometallurgical ore extraction processes do not generate polluting gases, particulates, or slags.

4. Technologically feasible processes have been or are being developed for recovery from sulfide ores by leaching with iron-oxidizing bacteria for the following metals: antimony, arsenic, cadmium, cobalt, copper, gallium, indium, lead, manganese, molybdenum, nickel,selenium, tellurium, tin, uranium, and zinc. Commercial exploitation has lagged in many cases because leaching rates are slow, or metal recovery from pregnant solutions is too costly.

5. Proven microbial leaching processes exist using acidophilic sulfur-oxidizing bacteria (which cannot oxidize iron) for the following metals: aluminum, arsenic, cadmium, chromium, copper lead, maganese, nickel, vanadium, zinc.

6. In bioleaching of ores, the action of microorganisms may be direct, indirect, or a combination of both actions.

7. Direct action involves direct enzymatic attack of the crystal lattice by the microorganisms and involves either an oxidation or reduction of the lattice component.

8. Indirect action involves generation of a product by the microorganism which reacts chemically with an ore causing its solubilization.

9. Some ores are resistant to leaching. Reasons are difficult to generalize, but include:

(1) one or more toxic or harmful components are in the ore or gangue
(2) the gangue may have significant adsorption capacity for leached metal ions, dimishing recovery of the metal ion

from solution

(3) the extracted metal ion may passivate the surface of the mineral

(4) the mineral component of the ore may be so finely disseminated in the gangue that it is not readily accessible to the leaching microbes

(5) some microbes cannot attack perfect crystal lattices -- imperfections in the lattice are needed to initiate the attack.

10. For commercial extraction of metals from ores and metal-containing wastes, either autotrophs or heterotrophs may be used. Autotrophs are generally favored because they do not require costly organic nutrients, although they exhibit slow growth rates. Heterotrophic activities applicable to mineral extraction are summarized in Table 13.

11. Bioleaching of copper from low grade ores and mine wastes by acidophilic, iron-oxidizing bacteria is a continuing practice. This type of leaching requires little manpower, no special equipment (except in recovery of metal from the pregnant solution), and is cost effective.

12. Bioleaching of uranium ores with acidophilic, iron-oxidizing bacteria is being commercially exploited.

13. Metal oxides, carbonates and silicates can be bioleached in the case of: chromium, iron, tin, antimony, aluminum, titanium, vanadium, and manganese. Commercial exploitation to date has been slow due to the slow rate of extraction and the low maximum amounts extracted.

14. Investigations are currently underway in Germany into the recovery of valuable metals such as arsenic, cadmium, chromium, copper, lead, nickel, vanadium, and zinc from industrial wastes

such as fly ash, slags, sludge, etc. Metal recoveries ranging from 30-100 percent have been reported depending on the nature of the material and the microbial process employed.

15. Once a metal is brought into solution from the ore by microbial means, it must be recovered by conventional cementation, solvent extraction, or electrolytic means.

16. Genetic engineering of metal-solubilizing and metal-concentrating bacteria is in its infancy. This is because the genetic properties of organisms like *Thiobacillus ferrooxidans*, *T. thiooxidans*, *Sulfolobus spp.*, *Leptospirillum ferrooxidans*, thermophilic thiobacilli, and others have not been clearly worked out. Plasmids have been found in *T. ferrooxidans*, but what they control remains to be discovered.

17. Although no standard procedures for bioleaching have yet been developed, four general processes are being developed:

(1) dump, heap, in-place
(2) batch
(3) continuous
(4) semi-batch/semi-continuous

Scale-up problems and modelling experiments for these are discussed, and a computer modelling program is available for use by subscribers to this study.

1.3.4 Technology of Microbial Metal Beneficiation

1. Bioleaching and ore beneficiation of gold and silver ores is receiving widespread attention. Recent experiments indicate that gold recovery by microbial beneficiation of ore concentrate is cheaper than conventional flotation - roast beneficiation.

1.3.5 Technology of Microbial Metal Concentration

1. Bacteria, algae, or fungi can be employed in metal concentration systems. The choice of organism depends on the source of the metal, the metal itself, whether the metal is the form of free ions, complexes, or precipitates, and which nutrients are present.

2. Microbes can concentrate certain metals from very dilute solutions or waste streams, in some cases concentrating them by several orders of magnitude relative to their concentration in solution (see Tables 7 and 8). Only a few cases of this have been commercially exploited. Such concentration processes are economically viable if

- (1) the process is relatively rapid
- (2) the process involves concentration from dilute solution by several orders of magnitude
- (3) the separation of the metals from the biomass is simple and inexpensive
- (4) the biomass is reuseable
- (5) the process of disposal of spent biomass is easy and inexpensive.

3. To be commercially attractive, a bioaccumulator (whether intact cell or isolated cell component) must

1. have a high selective affinity for metals
2. be readily and cheaply producible
3. be reuseable after elution of the concentrated metal
4. be chemically stable
5. be cheaply and safely disposable.

4. Certain bacteria will concentrate and deposit iron as magnetite crytals. The magnetite crystals may be produced en mass by culturing the bacteria which produce them in large

quantities. A patent has been issued for the commercial use of this process.

1.3.6 Technology of Miscellaneous Microbial Metal Interactions

1. On the average, sulfur compounds (inorganic and organic forms) constitute up to 7 percent of the coal in the U.S.A. Pyritic sulfur can be removed microbiologically in yields up to 97 percent if the coal is first pulverized and subjected to oxidative attack by acidophic, iron- and sulfur-oxidizing bacteria. This method is cheaper and simpler to operate than comparable chemical oxidation processes.

2. Rust removal and descaling by microbial means is technically feasible, but has not been exploited to date.

3. Metal powders can be produced from pregnant solutions generated by bioleaching of metallic ores. Copper powder can be produced by cementation with iron, or by reduction of cuprous cyanide with hydrogen. Copper and nickel powders can be produced by reduction in high pressure autoclaves.

1.4 RECOMMENDATIONS AND NEW BUSINESS OPPORTUNITIES

1.4.1 General Recommendations

(1) While a variety of microbes are capable of extracting metals from ores and are capable of concentrating metals from solution, in most instances little is known in detail about the kinetics, the biochemistry, and the physiology of these processes. Thus their applicability on an industrial scale must await the results of further research efforts which should be funded and pursued now.

The risk in investing in such R&D could be shared among interested companies, and its size could thus be significantly reduced for each participating company by forming and funding an R&D partnership which would perform the necessary R&D work in-house and through external contracts with special research firms and universities. Results of such work would be shared by all members of the partnership.

Gorham International Inc. is very interested in organizing and managing such an R&D partnership: subscribers should write for details.

(2) To be economically attractive, all microbial metal extraction and concentration processes must be kept simple. They should be designed to require simple equipment, cheap and available reagents, little manpower, and should they proceed at a satisfactory rate to recover the metal in a reasonable period of time.

(3) To improve the speed and yield of microbial extraction/concentration processes, engineered organisms should be designed and used. Such genetically engineered microorganisms should exhibit one or more of the following characteristics:

1. improved leaching capacity over a wild-type strain;
2. improved resistance toward the toxicity of the metals to be leached;
3. improved competitiveness toward the wild type microbial population in the leach process.

(4) To prevent unauthorized acquisition of genetically engineered cultures by competitors, it is recommended that strains be tagged with special genetic markers to identify them as coming from a specific source in case of legal disputes.

1.4.2 Initial Commercial Development Efforts

(1) Current industrial- scale applications of microbial extaction/concentration are limited to leaching low-grade ores, but the real promise of this technology exists in applying bioleaching to ore concentrates.

(2) It is recommended that industrial efforts be focused on the metal- sulfide, -oxide and -carbonate containing ores which are especially amenable to bioleaching, and to those ore wastes, slags, slimes, etc., that contain a variety of heavy metals, including very toxic ones. Microbes can also be successfully applied to beneficiation of certain ores, and it is recommended that this aspect of the technology be pursued vigorously at an early date.

(3) When considering the development and use of genetically engineered microorganisms, it is recommended that initial efforts be focused on bioleaching of ore concentrates and bioconcentrations of wastes and dilute solutions.

(4) It may be more efficient to accomplish metal accumulation with purified cell components or cell products from genetically engineered organisms. It is recommended that this route be examined and pursued even before direct use of engineered strains for metal accumulation is undertaken.

1.4.3 New Business Opportunities

(1) The economics and feasibility of bioleaching of ores containing precious strategic or heavy metals appears especially attractive for commercial exploitation within the short term. When selecting which specific metal/microbe combinations should be pursued, criteria should include questions about

1. whether more than one metal can be leached from the ore
2. whether the metal has high monetary value
3. whether the metal has strategic value.

(2) For dump and heap leaching, the only special equipment needed will be corrosion-resistant piping and pumps, and launders. For concentrate leaching, reactor vessels, separators, grinding equipment, electrolysis equipment and solvent extraction equipment will be needed. It is recommended that equipment and instrument producers work closely with those conducting R&D specifically on ore concentrate bioleaching, to help design and secure early patent rights on advanced equipment and instruments.

(3) For leaching processes involving chemolithotrophic bacteria, requirements for nutrient supplementation will be limited to sulfuric acid (or sulfur), and ammonium sulfate and/or phosphate. It is unlikely that a major business opportunity exists for the supply and maintenance of nutrients for these processes.

(4) For leaching processes involving heterotrophs, organic carbon and energy sources will be needed (glucose, sulfite waste liquor, sewage etc.) at concentrations at 1-3 percent (wt/vol). Nitrogen requirements may be met with inorganic compounds (urea, ammonium sulfate) or organic nitrogen (protein hydrolysates, sewage, agricultural wastes etc.). Supplying and monitoring these nutrients at mine locations could represent an important new business opportunity when these processes are commercially exploited.

(5) Microorganisms can be used as aids in locating specific types of ore deposits. This technique (bioprospecting) should be exploited and utilized on a far larger scale than heretofore as a supplement to conventional prospecting methods.

(6) Utilization of engineered strains by ore processors may be as a test-tube size culture, which the ore processor then would

have to build up to inoculum-size by cultivation. Alternatively, the genetic engineering company or a special distributor might undertake to supply strains in quantities ample to innoculate the ore body. This special business opportunity should be pursued once the satisfactory development of an engineered strain has proceeded to a semi-commercial scale. Greatest potential for this exists in leaching ore concentrates in special reactors.

2.0 INTRODUCTION

2.1 A BRIEF HISTORY

The concept of using microbes as metal extractors of ores dates back to the early 1950's. In 1954, Bryner et al. published a paper in which they described experiments in which acidophilic thiobacilli from mine drainage of Kennecott's Bingham Canyon open pit mine were made to oxidize iron pyrites and copper sulfides including covellite (CuS), chalcocite (Cu_2S), bornite (Cu_5FeS_4) and tetrahedrite ($Cu_8Sb_2S_7$). The organisms were identified as Thiobacillus ferrooxidans and T. thiooxidans by Bryner and Jameson in 1958, the same organism shown earlier by Colmer and Hinkle (1947), Colmer et al. (1950), and Temple and Colmer (1951) to be the chief cause of acid mine drainage from bituminous coal mines. T. ferrooxidans was shown to oxidize free sulfur, ferrous iron, iron pyrites, molybdenite (in the presence of pyrite) and several copper sulfide minerals. T. thiooxidans was shown to oxidize free sulfur but not sulfide minerals. These observations led in 1958 to the issuance of the first patent to Zimmerley et al., assignors to Kennecott Copper Corporation, on a cyclic leaching process employing acidophilic iron - oxidizing bacteria. This patent covered the leaching of metals from metal sulfide- and oxide- ores with the acid ferric sulfate lixiviant generated by "motile, non-sporforming, iron-oxidizing, autotrophic, rod bacteria which are tolerant to the metallic constituent or constituents to be extracted." In the process, the role of the bacteria was described as generating ferric sulfate and sulfuric acid from dissolved ferrous iron. The acid ferric sulfate was seen as the agent directly responsible for solubilizing the metal sulfide (MS), e.g.,

$$MS + 2Fe^{3+} = M^{2+} + 2Fe^{2+} + S^{o}. \qquad (1)$$

It was, however subsequently shown that the bacteria could also oxidize the metal sulfides by direct attack (e.g., see Silverman and Ehrlich, 1964; Torma, 1977). It was also subsequently shown that other acidophilic iron- and sulfur-bacteria, some thermophilic, may participate in the leaching process (Brierley and Lockwood, 1977). The initial Kennecott patent led to the issuance of a series of additional patents (see Table 1).

The practice of leaching copper-containing ores through the action of acid ferric sulfate solutions predates the discovery of acidophilic thiobacilli and their role in this process by more than 200 years. The first major application appears to have been at Rio Tinto, Spain, in about 1752. At that time, ore piles were watered and the dissolved copper recovered by treatment with metallic iron (Sheffer and Evans, 1968). According to Sullivan (1930), Siemens and Halske (Germany) in 1890 leached roasted copper ore containing chalcocite with hot, acid ferric sulfate solution. Sullivan (1930a) also mentions leaching of concentrator tailing sands and flue dust at Cananea (Mexico) and a mixed copper oxide and copper sulfide ore by the New Cornelia Copper Company in Ajo, Arizona. The first large-scale leaching in the U.S. of mixed- sulfide copper ore was introduced by the Inspiration Copper Company in 1926 (Van Arsdale, 1926). The common belief of early ore processors was that leaching as practiced in the field was a purely chemical process. Indeed, the chemistry of the oxidizing action of acid ferric sulfate was studied in detail by J.D. Sullivan in the late 1920's (Sullivan, 1930a, 1930b, 1931). He showed that acid ferric sulfate solution was a powerful oxidant which dissolved the copper in chalcocite, covellite, and bornite and converted the sulfide to sulfur. In the field, sulfur does not, however, accumulate in significant amounts during leaching of sulfide ores, which should have been a clue that something more was happening.

The early work of Bryner and coworkers (Bryner et al., 1954, Bryner and Anderson, 1957; Bryner and Jameson, 1958) showed that it was possible to leach metal sulfide ores microbially and led to leaching studies of a variety of other metal sulfides and to the development of methods for microbial leaching of ore

Table 1. Patents on microbial metal extraction

Authors	Assignee	Year	Patent no.	Topic
Zimmerley, S.R., D.G. Wilson, and J.D. Prater	Kennecott Copper Corp.	1958	US 3,333,953	Cyclic leaching process employing iron-oxidizing bacteria
Mayling, A.A.	--	1966	Can. 744,701	Using bacteria to keep ferric chloride in solution oxidized for leaching.
Duncan, D.W. and C.J. Teather	British Columbia Research Council, Vancouver, B.C.	1966	US 3,266,889	Method for extracting metals from sulfide ores using bacteria and accelerating agent
Goren, M.B.	Kerr-McGee Oil Industries, Inc.	1966	US 3,252,756	Oxidation and recovery of vanadium values from acidic aqueous media
Zimmerley, J.R., and J.D. Prater	Kennecott Copper Corp.	1967	US 3,333,953	Process and apparatus for the precipitation of substances from solution using solid precipitants
Zimmerley, J.R., E.E. Malouf, J.D. Prater, and Schillinger	Kennecott Copper Corp.	1967	US 3,330,650	pH adjustment controlled-iron-content, cyclic leaching process for copper-bearing rock materials
Duncan, D.W., C.C. Walden, and P.C. Trussell	British Columbia Research Council, Vancouver, B.C.	1967	US 3,305,353	Accelerated microbial ore extraction process
Mayling, A.A.	Roan Selection Ltd., Vancouver, B.C.	1969	US 3,455,679	Bacterial leaching of ores with an alkaline matrix

Table 1 (continued)

O'Connor, L.T., J.A. Brierley, and R.B. Bhappu	--	1972	US3,679,397	Bacterial leaching of metallic constituents from metallurgical materials
Watanabe, A., T. Uchida, S.Furuyama, and J. Koide		1972	Japan 7,214,602	Recovery of valuable metals from sulfide ores by bacteria
McElroy, R.O., and D.W. Duncan	British Columbia Research Council, Vancouver, B.C.	1974	US 3,856,913	Copper extraction by rapid bacteriological process utilizing silver salt
Tsuchiya, H.M.		1975	S. Afr. 7,402,879	Bacterial leaching of copper an nickel
Mikulski, T., A. Kevemcska, and K. Czermak		1977	Poland 93,356	Bacterial leaching of copper ores, collector concentrates, shaft dust, and slimes
Torma, A.E.		1978	Can. 1,023,947	Selective bacterial cyclic leaching process
Bagdash, Z., and J. Starosta		1978	Poland 193,508	Non-ferrous metal sulfide mineral alkaline raw materials for bacterial extraction
Torma, A.E.	Centre de Recherche Minerale, Ministere des Recherches Naturelles du Quebec	1978	Can. 203,547	Bacterial leaching of Cu, Cd, Zn and Pb sulfide-containing ores
Richardson, F.J.		1981	US 4,256,485	Enzyme oxidation of sulfides in minerals
Pooley, F.D.		1982	UK 2,097,369A	Removing excess sulfur by roasting prior to bacterial leaching to improve metal recovery

Table 1 (continued)

Nandi, M.M. and B.R. Sant	1982	India 150,466	Microbial recovery of copper from copper ores. (Note: stimulate bacteria by feedin initially!)
Solozhenkin, P.M., L.L. Lynbavina, O.G. Levchenko, T.I. Rastopshina	1983	USSR 990,842	Recovery of copper from hard-to-concentrate ore raw material

concentrates (see Silverman and Ehrlich, 1964; C.L. Brierley, 1978; Torma, 1977; Murr, Torma, and Brierley, 1978; Cripps, 1980). However, large-scale, microbial leaching is up to now being practiced only on low-grade copper and on uranium ores in the western world. Microbial uranium leaching was pioneered in uranium mines at Elliott Lake, Ontario, Canada (MacGregor, 1966; Zajic, 1969; Brierley, 1982; Cripps, 1980).

The observation that acidophilic iron bacteria can promote ore extractions led others to investigate the ability of neutrophilic, heterotrophic bacteria and fungi to promote ore extraction. Among the first to examine this approach were Perkins and Novielli (1962), who examined manganese extraction from manganese ores; Pares (1964a,b,c), who examined extraction of copper, iron, and gold from laterite and clay; Lyalikova and Mokeicheva (1969), who examined extraction of gold from gold ore; Wenberg et al. (1971) and Hartmannova and Kuhr (1974), who examined the extraction of copper from carbonate-rich copper ores; and Silverman and Munoz (1971), who examined titanium extraction from some igneous rocks.

The idea of using microorganisms to concentrate metals can be traced back at least as far as the middle 1960's when Walter N. Ezekiel reported that the U.S. Bureau of Mines was investigating the use of bacteria to concentrate hafnium and zirconium from solution (Science News, 1966). The idea was based on various observations that bacteria and other microorganisms can accumulate metals by concentrating them manifold over their concentration in the environment (e.g., Somers, 1963; Engel and Owen, 1970). The various mechanisms by which such accumulations occur began to be understood only more recently (see Norris and Kelly, 1977; Brierley and Brierley, 1983). Practical applications began to be suggested in the 1960's (e.g., Friedman and Dugan, 1968).

2.2 CONVENTIONAL METAL EXTRACTION/RECOVERY PROCESSES

In preparation for metal extraction, ores have to undergo ore dressing, a beneficiation process. Relatively rich metal ores, that is, ores with an economically favorable metal-to-gangue ratio, are sent directly to an ore crusher and thence to rod and/or ball mills for further comminution. Metal values may then be separated from the gangue by gravity separation, magnetic separation, or froth flotation. The following metal-containing minerals may be separated from gangue by froth flotation: metal sulfides, heavy metal oxides, metal silicates, metal carbonates, and inert minerals. In froth flotation, metal mixtures, such as those of copper and molybdenum sulfides, may be separated from each other by the use of different flotation (surface active) agents. The residual gangue constitutes the tailings and must be disposed. The metal values constitute an ore concentrate from which the metal must be separated. This may be accomplished pyrometallurgically by smelting or hydrometallurgically by chemical and/or microbiological leaching followed by electrochemical separation, or solvent extraction and reduction to the metallic state .

Pyrometallurgy involves smelting of the enriched ore, including chemical reactions to separate metal values from each other and/or the slag, and collecting the metal in reduced form. Further refining of the metal may be required. Pyrometallurgy is an energy-intensive process and requires rich or enriched ore to be economical. The process may contribute to air pollution if flue gases are allowed to escape untreated.

Hydrometallurgy involves the extraction of metals from ore by a chemical or biochemical solution process. The chemical solution process may, for example, involve cyanidation as for gold and silver recovery, acid treatment as in leaching of copper oxide- or uranium-ores, or in oxidation as when acid ferric sulfate reacts with metal sulfide ore. The dissolved metals may be recovered electrochemically in relatively pure form, but this is an energy-intensive process. Solvent extraction and precipitation are alternative methods of metal recovery from solution. Chemical leaching can contribute to water pollution if not carefully controlled. Biohydrometallurgy is discussed in detail in section 2.3.

The extraction of certain metals may require special treatment of ores as in the case of bauxite for aluminum production. The bauxite may be freed from iron impurities by treatment with lye, leaving ultimately a fairly pure alumina (Bayer process). The alumina is reduced to aluminum by fused-salt electrolysis. This is also an energy intensive process.

For more detailed discussion of extractive metallurgy, the realder is referred to Rosenquist (1974), and Pryor (1965).

2.3 RATIONALE FOR MICROBIAL METAL EXTRACTION/RECOVERY

2.3.1 Extraction

In ore processing, microbes can aid in (1) beneficiation, (2) metal extraction, and (3) metal recovery from solution. Microbial beneficiation of ore can be achieved by selectively leaching away undesirable ore constituents. Microbial metal extraction is achieved by solubilization of the metal values in the ore. It must be stressed, however, that in general, microbes are incapable of reducing metallic compounds to the metallic state. One exception is the reduction of mercuric mercury to metallic mercury, but in nature this involves only small quantities of mercury at very low concentrations of Hg^{2+}, so that this biological process can be harnessed industrially only if it can be artificially amplified (by genetic engineering) and if the biological system can be made more resistant to toxic Hg^{2+}.

Another exception is the reduction of oxidized selenium or tellurium to elemental selenium or tellurium, respectively.

From an economic standpoint, microbial metal extraction is attractive if:

1. the metal value of the ore is too low for conventional extraction methods, i.e., the metal value/gangue ratio is too unfavorable (e.g., for copper sulfide ores below 0.4%);

2. the metal extraction cannot be easily achieved by purely chemical/physical methods;

3. the non-biological method of metal extraction is too costly because

    a. it is too energy-intensive
    b. special reagents needed in the extraction are too costly
    c. special processing equipment is too costly;

4. the conventional process produces air/water pollutants whose disposal into the environment is too costly to control.

To be viable, microbial metal extraction processes must involve simple equipment, require cheap, easily available supplemental reagents, be operable with minimal manpower, proceed at a sufficient rate so that economic quantities of metal can be recovered from large quantities of ore in reasonable time.

2.3.2 Metal Concentration

Metal concentration by microbes can aid in

1. the accumulation of metals for exploitation from very

dilute solution where chemical or physical methods would be uneconomical;

2. the recovery of metals from dilute waste streams from metal refineries and other metal-utilizing industries;

3. the removal of metal toxicants from polluted waters and waste streams for water purification.

Microbial metal concentration may be achieved through extracellular precipitation, by adsorption or other binding processes to a cell surface, or by intracellular accumulation. Such processes are economically attractive if

1. the process is relatively rapid,

2. the process involves concentration from dilute solution by several orders of magnitude,

3. the separation of the metals from the biomass is simple and inexpensive,

4. the biomass is reuseable,

5. the process of disposal of spent biomass is easy and inexpensive.

As in metal extraction, any equipment needed for running the process should be simple. Biomass production must be inexpensive, i.e., the nutrients required must be readily available and cheap. Autotrophic microorganisms (e.g., cyanobacteria, algae) are more desirable than heterotrophs (fungi, organic matter-consuming bacteria) when growing cultures are to be employed.

2.4 CLASSIFICATION OF MICROBE/METAL INTERACTIONS

Microbes interact with metals in different ways. Some of these interactions are large-scale and some small-scale. Large-scale enzymatic interactions involving metal oxidation or reduction are restricted to prokaryotes (bacteria, cyanobacteria). This has to do with their unique cell organization, more specifically the location of requisite enzymes in the cell envelope (Ehrlich, 1978). Small-scale enzymatic metal interactions are found in both prokaryotes and eukaryotes (algae, fungi, protozoa). This is because these reactions are assimilatory in nature or involve detoxification mechanisms. Large-scale and small-scale, non-enzymatic metal interactions can be performed by both prokaryotic and eukaryotic microorganisms.

Microbe/metal intreractions can be classified as follows:

A. **Metal oxidation**

a. Enzymatic

1. Some prokaryotes can derive metabolic energy (adenosine 5'-triphosphate, ATP) from the oxidation of certain metals

   ex., $Fe^{2+} = Fe^{3} + e^{-} + ATP$ (T. ferrooxidans, Sulfolobus spp., etc.);

   $Cu_2S = Cu^{2+} + CuS + e^{-} + ATP$ (T. ferrooxidans);

   $Mn^{2+} + 2H_2O = MnO_2 + 4H^{+} + 2e^{-} + 2ATP$ (Arthrobacter, Oceanospirillum, etc.).

2. Some autotrophic prokaryotes can use the reducing power liberated in the oxidation of certain metals to reduce pyridine nucleotide (NAD, NADP).

ex., $2Fe^{2+} + NADP^+ + 2(H) = 2Fe^{3+} + NADPH + H^+$

$NADPH + H^+ + CO_2 = (CH_2O) + NADP^+$

3. Some microorganisms can oxidize metals to detoxify their environment.

ex., $Mn^{2+} + H_2O_2 \xrightarrow{catalase} MnO_2 + 2H^+$ (*Metallogenium, Leptothrix pseudoochracea, Arthrobacter siderocapsulatus*). H_2O_2 is the toxicant.

b. Non-enzymatic

1. Some microbes can oxidize metals with a metabolic product they produce

ex., $Mn^{2+} + 2H_2O$ + oxidized compound = $MnO_2 + 4H^+$ + reduced compound (*Streptomyces* sp.)

2. Cyanobacteria and algae can oxidize metals by production of alkali and/or oxygen in photosynthesis

ex., $2HCO_3^- = 2CO_3^{2-} + 2CO_2 + H_2O$

$CO_3^{2-} + H_2O = HCO_3^- + OH^-$

$CO_2 + H_2O = (CH_2O) + O_2$

$2Fe^{2+} + 6OH^- + 1/2\ O_2 + 2H^+ = 2Fe(OH)_3 + H_2O$

B. Metal Reduction

a. enzymatic

1. Some prokaryotes can use metallic compounds as terminal electron acceptors in respiration in place of oxygen

   ex., $Fe_2O_3 + 5e^- + 6H^+ = Fe^{2+} + 3H_2O$ (Clostridium butyricum, Bacillus polymyxa),

   $MnO_2 + 2e^- + 4H^+ = Mn^{2+} + 2H_2O$ (Bacillus 29, Vibrio sp., etc.),

   $MoO_4^{2-} + 2e^- + 4H^+ = MoO_2 + 2H_2O$ (Sulfolobus sp.).

2. Most microbes living at or near neutral pH require iron and can take it up only if chelated by a siderophore.. Once taken up, the iron is released from its siderophore by reduction. In a few instances the siderophore may be hydrolyzed before the iron is reduced and released from the siderophore.

   ex., ferrisiderophore + e^- = desferrisiderophore + Fe^{2+} (see also items C and F below).

3. Metallic ions may be reduced to a less toxic form

   ex., $Hg^{2+} + 2e^- = Hg^o$

b. Non-enzymatic

1. Oxidized forms of metals may be reduced by metabolic products which act as reductants.

   ex., $MnO_2 + HCOOH + 2H^+ = Mn_{2+} + CO_2 + 2H_2O$
   $MnO_2 + HOOCCOOH + 2H^+ = Mn_{2+} + 2CO_2 + 2H_2O$

$$2Fe^{3+} + 3H_2S = 2FeS + 6H^+ + S^o.$$

C. Metal Chelation

1. by production of metabolic products which can act as chelators

   ex., a. siderophores of the hydroxamate, catechol, or mixed type (affinity coefficient for FeIII is $K_a = 10^{38}$ to 10^{52}; affinity coefficient for FeII is $K_a = 10^8$);

   b. citrate, chelator of FeIII, Mg^{2+}, Ca^{2+};

   c. amino acid chelators of Mn^{2+}, etc.

D. Metal Methylation

Some bacteria have the ability to methylate Hg^{2+}, Sn^{2+}, Pb^{2+}, Cd^{2+}, $PtCl^{6-}$, AsIII, Se^{2-}, Te^{2-} (Hallas et al., 1982, Iverson and Brinckman, 1978; Summers and Silver, 1978).

ex., $Hg^{2+} \xrightarrow{CH_3B_{12}} CH_3Hg^+ \xrightarrow{CH_3B_{12}} (CH_3)_2Hg$

E. Metal Sorption

1. Bacterial cell wall

It has been shown that specific components of the bacterial cell wall can bind metal ions. In Bacillus subtilis it is mainly the peptidoglycan, whereas in B. licheniformis it is teichoic and teichuronic acids (Beveridge and Murray, 1980; Beveridge et al., 1982).

2. Bacterial slimes

Slime produced by the bacterium Zoogloea ramigera has been shown to be able to bind metal ions (Friedman and Dugan, 1968).

F. **Metal Transport**

Microorganisms accumulate some metals intracellularly, in part because they need these metals for enzyme function. In both prokaryotic and eukaryotic systems, special transport systems exist in the cell envelope to pull the metals in ionic form through the cell membrane into the cell interior. Some of these transport systems are relatively non-specific and may transport several different metal ions. The affinity for different ions likely differs, however. Different ion species transported by the same transport system will compete with each other for translocation, depending on their respective concentrations. Both cationic and anionic species may be transported although by different carriers. In some cases, energy has to be expended by a cell to transport a metal into its cytoplasm. Because some of the transport systems are relatively non-specific, they may carry toxic ions into cells (Jasper and Silver, 1977; Silver and Jasper, 1977). In the case of iron, special transport mechanisms exist in prokarykotes and eukaryotes to take up ferric iron chelated by appropriate siderophores (Lankford, 1973). The iron is released from siderophores by reduction after it has passed through the plasma membrane in chelated form (Lankford, 1973). In some cases, the siderophore has to be hydrolytically cleaved before the iron is reduced and released (Lankford, 1973).

Although transport systems which are driven through consumption of metabolic energy may cause intracellular accumulation against a concentration gradient over several orders of magnitude, the quantities of corresponding metal ions involved per cell are relatively small.

G. **Inhibition of Metabolism**

Most metals are toxic above critical concentrations. This is true even of metals that may be essential nutritionally as, for instance, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, and Zn. The reasons for metal toxicity are varied. They may be summarized as follows.

1. binding of a metal ion like Hg^{2+} to a functional group of an enzyme or carrier protein, such as an -SH group, and thus interfering with enzyme catalysis or membrane transport, respectively.

2. Blocking metal incorporation into an enzyme protein as in the interference of tungstate in molybdenum incorporation into nitrate reductase.

3. Interfering with uptake of an essential metal because of competition by a non-specific carrier system.

4. By reacting with a nutrient molecule to form a compound which the cell cannot absorb.

5. By reacting with the genetic substance (DNA) of the cell and preventing its proper replication, as in the case of $CrO_4{}^{2-}$ which cross-links DNA.

Microorganisms can develop resistance to toxic metals by genetic modification (chromosomal mutation or specific plasmid acquisition or modification). The result may be decreased membrane permeability to the metal, perhaps by carrier protein modification or elimination. The result may also be enzyme modification such that the protein no longer has affinity for the toxic metal, or elimination of the sensitive enzyme protein and by-passing the reaction it catalyzed in the cell.

Some microorganisms may also protect themselves against metal toxicity by chemically modifying the metal to eliminate or lessen its toxicity, as by methylation, precipitation as an insoluble compound, or by volatilization. CH_3Hg^+ is adsorbed to clays and can thus be taken out of circulation. Metals that may be biomethylated include Hg, Pb, Sn, Pt, As, Se, and Te (Iverson and Brinckman, 1978). Heavy metals such as Cu, Co, Ni, Zn, etc. can be precipitated as sulfides by biogenic H_2S from sulfate reduction by sulfate-reducing bacteria. Dimethylmercury, other methylated metals, and metallic mercury produced by bacteria are volatile and not water soluble and, hence, pass readily into the atmosphere.

2.5 REGULATORY IMPLICATIONS

Smelting of ores such as sulfide ores generates gases such as oxides of sulfur and CO_2 and particulates such as sulfate, arsenates, and silicon salts, whose uncontrolled release into the atmosphere causes air pollution. Solid residues (slag) when dumped without provisions for containment of leachate can release toxic metals into the environment.

Biohydrometallurgical ore extraction eliminates the possibility of generating polluting gases and particulates. It also eliminates the production of slag, although other possibilities exist for contaminating the environment with toxic metals from the leachate if proper provisions of containment are not made.

Toxic metals may also be introduced into the environment from industries other than those involved in ore extraction or metal refining. Examples are domestic waste treatment plants, chemical industry, petroleum industry, etc. Metal concentration and separation by biomass may provide an economical solution to their problem.

The Office of Technology Assessment of the United States Congress has expressed some concern about the potential future use of genetically engineered microorganisms in heap-, dump-, and in-place leaching (Office of Technology Assessment, 1981). It fears that releasing such microorganisms into the environment might cause "ecological disruption and liability if damage occurs to the environment or human health." Such fears would seem to be largely unfounded, however. Bacterial strains engineered for more efficient leaching are most likely less competitive vis-a-vis wild-type strains and would run a great risk of being rapidly overgrown by the native organisms unless specially engineered to cope with such competition (a very difficult task at best). As will be explained in section 5.2.2, engineered organisms will find their widest application in the leaching of ore-concentrates in reactors, where competing and interfering microorganisms can be held in check. In such controlled applications of engineered organisms, it is an easy task to prevent them from being introduced into the general environment. Microbes used in leaching, especially chemolithotrophic bacteria, do not represent a health hazard. Any heterotrophic bacteria or fungi employed should obviously not include any with potentially pathogenic traits. The same generalizations apply to microbes used for concentrating metals from solution.

3.0 TECHNICAL REVIEW AND ASSESSMENT

3.1 EXTRACTION OF METALS FROM INSOLUBLE MINERALS, ORES, ASHES, AND SLAGS

3.1.1 Proven and Commercial Microbial Leaching Processes

3.1.1.1 Acidophilic, Iron-Oxidizing Bacteria

According to current estimates, about 10 to 20% of recently produced copper in the U.S. were obtained by microbial leaching of low-grade sulfide ores (Lundgren and Malouf, 1983). This involved generally dump- and in-place leaching. Significant amounts of uranium are also currently extracted microbiologically in the U.S. and Canada.

Kelly et al. (1979) have divided leaching processes of sulfide ores into contact and remote systems. Contact systems by definition involve large-scale heap and dump systems in which chemical as well as bacterial oxidation occurs through intimate contact with the minerals. Contact processes can also involve vat leaching of high-grade ore and ore concentrates. Remote leaching systems involve biogeneration of lixiviant at a physically distinct site and then pumping the finished lixiviant unto the dump or heap, or into the ore body for extraction of the metal values. The lixiviant may be acid ferric sulfate solution. The advantage of remote leaching is said to be that the actual leach process may not favor bacterial activity because toxic products may be formed in the leaching process (UO_2^{2+}, MoO_4^{2-} which are toxic to some strains of Thiobacillus ferrooxidans), and because physical conditions of leaching, such as elevated temperature, may be preferred which are inimical to the bacteria.

On close examination, these distinctions break down, however. Since leaching processes are cyclic, and since organisms active in leaching may be found in all parts of the leach cycle, although not in equal numbers, it is impossible to separate remote leaching from contact leaching. Leach dumps, leach heaps, broken-up ore bodies, etc. are constantly being reinoculated by the bacteria in the circulating lixiviant. The argument that production of toxic products from some ore may prevent contact leaching by bacteria also cannot be supported. It has been shown, for instance, that while T. ferrooxidans does, indeed, find MoO_4^{2-} toxic (Tuovinen and Kelly, 1972) and, therefore, does not oxidize pure molybdenite (MoS_2), it will oxidize it in the presence of $FeSO_4$ or FeS_2 (Bryner and Anderson, 1957). The reason it can oxidize MoS_2 in the presence of Fe^{2+} or FeS_2 is that the molybdate titer is held at non-toxic levels by its precipitation by ferric iron. It has also been shown that strains of T. ferrooxidans resistant to uranium (VI) exist. Indeed, some can use uranium (IV) as an energy source (Dispirito and Tuovinen, 1981, 1982a, 1982b). Thus uranium leaching, like copper leaching, may proceed by contact as well as remote leaching at the same time.

Copper and uranium ore leaching are not the only extraction processes that can be promoted by acidophilic iron bacteria. Zinc, nickel, molybdenum, lead, cobalt, cadmium, arsenic, antimony, gallium, indium, thallium, tin, etc. can be extracted from appropriate sulfide ores by acidophilic iron oxidizers in processes that have been reported to be technologically feasible (Table 2).

Not only may sulfide ores or sulfide ore concentrates be bioleached, but by-products of metal refining may also be bioleached by T. ferrooxidans. For instance, Corrick and Sutton (1968) succeeded in leaching lead blast furnace matte. Ebner (1977, 1978) reported different degrees of success in leaching a jarosite by-product of zinc production, sulfidic dust

Table 2. Technologically feasible processes for recovery of metals other than Cu or U from sulfide ores by leaching with iron-oxidizing thiobacilli

Metal	Type of mineral	Type of material	Reference
As	As_2S_3	Museum-grade orpiment	Ehrlich, 1963
	$FeS_2.FeAs_2$	Museum-grade arsenopyrite	Ehrlich, 1964
	$3Cu_2S.As_2S_5$	Museum-grade enargite	Ehrlich, 1964
	$FeS_2.FeAs_2$	Arsenopyrite	Kulebakin et al., 1974
Cd	CdS	Cd-, In-, Tl-, Ga-bearing sphalerite	Lylikova and Kulikova, 1965
	CdS	Synthetic CdS	Torma et al, 1974
Co	CoS	Synthetic CoS	Torma, 1971; Torma et al., 1974; Groudev, 1981
Ga	Ga_2S_3	Gallium-bearing chalcopyrite concentrate; synthetic Ga_2S_3	Torma, 1978
	Ga_2S_3	Tl-, Ga-, In-, Cd-bearing sphalerite	Lyalikova and Kulikova, 1965
In	InS	Tl-, Ga-, Cd-, In-bearing sphalerite	Lyalikova and Kulikova, 1965
Mn	MnS and $MnCO_3$	Alabandite and rhodochrosite	Mao et al., 1979
Mo	MoS_2	Ore concentrate	Bryner and Anderson, 1957; Bryner and Jameson, 1958; C.L. Brierley, 1974
Ni	NiS	Synthetic NiS	Hendy and Rogers, 1978; Torma, 1971; Torma et al., 1974
	NiS	NiS, Cu_2S, FeS_2 mixture; ilmenite-magnetite ores	Jagiello and Starosta, 1981;

Table 2 (continued)

Ni	NiS	Mixed ore: pentlandite with Ni, Cu, Co	Dutriziac and MacDonald, 1974
Pb	PbS	Galena: Tekli deposit	Stukanov, 1978
	PbS	Galena mixed with chalcopyrite	Kingma and Silver, 1980
	PbS	Mixed ore (Pb-Zn)	Ilayletdinov et al., 1977
Sb	Sb_2S_3	Stibnite	Torma and Gabra, 1977
Sn	SnS	Synthetic stannite, keskerite, stannodite, herzenbergite, beridsite, and natural tin minerals: stannite, cassiterite, valanoffite	Teh and Schwartz, 1981
Zn	ZnS	Synthetic ZnS	Torma et al., 1974
	ZnS	Ore concentrate	Brynesteyn and Duncan, 1971
	ZnS	Mixed ore (Pb-Zn)	Ilyaletdinov et al., 1977; Rozek et al., 1981

concentrate from a chemical copper extraction process, oxidic fly ash from a pyrite roasting process, and a basic slag from a lead smelting process. Szolnoki (1977) leached red mud, and fly ash from petroleum burning power stations. He recovered most of the aluminum from red mud by using biologically produced acid ferric sulfate at 60 degrees Celsius, and he recovered about 50% of the vanadium and nickel in the fly ash.

3.1.1.2 Proven Microbial Leaching Processes Using Acidophilic Sulfur-Oxidizing Bacteria Which Cannot Oxidize Iron

In the field, acidophilic, iron oxidizing bacteria are usually accompanied by Thiobacillus thiooxidans, an acidophilic, chemolithotrophic, sulfur-oxidizing bacterium which cannot oxidize iron. It can oxidize various forms of reduced sulfur but not metal sulfides to generate energy for CO_2 assimilation. It oxidizes elemental sulfur to sulfuric acid according to the following reaction

$$S^o + 3/2\ O_2 + H_2O = H_2SO_4.$$

It can form enough sulfuric acid from an excess of sulfur to lower the pH of its medium in a batch culture to 1.0 or slightly lower. This acid can be very effective in leaching certain ores (Table 3). T. thiooxidans has been shown to extract Cd^{2+} from CdS in the presence of added S^o (Norris and Kelly, 1978). It was also shown to solubilize Zn, Cu and As from jarosites, and oxidic fly ash, and basic slag from a lead smelter in Starkey's sulfur medium (Ebner, 1978). Similar findings have been reported by Bosecker (1983) with regard to acid-leaching of fly ash, alkaline soda slag, sludge, and tailings from galvanic and flotation processes. Recoveries included Zn, Cu, Ni, V, and Cr, depending on the composition of the waste material. Burba and Schaefer (1981) leached Cd, Cu, Mn, Pb, and Zn from industrial wastes with T. thioooxidans. They recovered some of these metals

Table 3. Ore leaching with *Thiobacillus thiooxidans*, which generates sulfuric acid in sulfur medium

Metal leached	Mineral or ore	Reference
Cr	Chromite ore	Ehrlich, 1983b
Ni	Pentlandite, nicollite Harzburger gabbro	Bosecker, 1977
U	Uranium ore	Bosecker and Wirth, 1980; Ebner and Schwartz, 1973
Zn	Marmatite	Khalid and Ralph, 1977

after solution by adsorption to cellulose. Ehrlich (1983b),on the one hand, was able to extract only limited amounts (<10%) of Cr and Fe from low-grade chromite ore with T. thiooxidans in Waksman's sulfur medium, but, on the other hand, recovered most of the Cr, Fe, and Ni from a sulfidized superalloy scrap. Wong et al. (1982) obtained poor solubilization of Cr from chromite ore with T. ferrooxidans, whether FeII or sulfur were used as energy source. Vanadium has been extracted from flexicoker coke with T. thiooxidans in the presence of added sulfur (Sullivan et al., 1980; Jack et al., 1980). This coke is a by-product from the treatment of Athabasca Tar Sands.

The principle of acid leaching can also be applied by the use of other microorganisms . Szolnoki (1975) has described the leaching of aluminum (Al^{3+}) from red mud, a waste product of aluminum production, with acidic ferric sulfate formed by T. ferroxidans. This ability of biologically produced acid ferric sulfate to mobilize aluminum was previously recognized in the patent by Zimmerley et al. (1958). Mehta et al. (1979) reported that Penicillium simplicissimus grown in glucose-containing medium with low-grade aluminum-bearing rock particles (basalt) leached almost 20% of the available aluminum in 30 days. The aluminum derived mostly from mineral phases containing olivine and plagioclase.

3.1.2 MICROBIOLOGY OF CHEMOLITHOTROPHIC SYSTEMS

3.1.2.1 Types of Microorganisms and Aspects of Their Physiology Pertinent to Leaching

The microorganisms active in the leaching of metal sulfide ores and uranium ores are all acidophilic bacteria (Table 4), i.e. bacteria that grow at pH values between 1.5 or slightly lower and about 5. Some grow at mesophilic temperatures, i.e.,

Table 4. Acidophilic chemolithotrophs active or having potential for activity in ore leaching

Temperature range preference	Name of organism	Opt. growth temp.	Opt. pH range	Special physiological traits	References
Mesophilic	*Thiobacillus ferrooxidans*	ca. 25 C	2.0-3.5	Oxidizes Fe^{2+}, S^{o}, $S_2O_3^{2-}$, U(IV), metal sulfides, CuSe	Buchanan and Gibbons, 1974
	Thiobacillus thiooxidans	28-30 C	2.0-3.5	Oxidizes H_2S, S^{o}, $S_2S_3^{2-}$ to sulfuric acid; does not oxidize metal sulfides	Buchanan and Gibbons, 1974
	Leptosprillum ferrooxidans	30 C	3.0	Oxidizes Fe^{2+} but not S^{o}.	Balashova et al., 1974
	Sheffield isolate	20 C	2.5	Oxidizes Fe^{2+}; becomes encrusted with oxides of iron	Cameron et al. 1981
Thermophilic	*Sulfolobus acidocaldarius*	70-75 C	2-3	Oxidizes Fe^{2+}, S^{o}; reduces FeIII anaerobically	Zillig et al., 1980
	Sulfolobus brierleyi	70 C	2.0	Oxidizes Fe^{2+}, S^{o}, MoS_2, $CuFeS_2$, reduces MoS_2 anaerobically	Zillig et al., 1980
	Sulfolobus solfatarius	70 C	3.5-5.0	?	Zillig et al., 1980

Table 4 (continued)

Thiobacillus spp.	55 C	1.4-3.0	Oxidize Fe^{2+}, S^0, metal sulfides; need trace of yeast extract for growth in lab culture	J.A. Brierley, 1978; Brierley and Brieley, 1978
Sulfobacillus thermsulfidooxidans	50 C	1.9-2.4	Oxidizes Fe^{2+}, S^0, forms endospores	Golovacheva an Karavaiko, 197

in a range around 25 degrees Celsius, others at thermophilic temperatures, in a range around 55 or 70 degrees Celsius. They are chemolithotrophs in that most obtain their carbon from CO_2 and their energy for growth from the oxidation of ferrous iron, reduced sulfur compounds, and in two cases, from uranium (IV) and selenide. Exceptions are _Thiobacillus thiooxidans_, which can get its energy only by oxidizing reduced sulfur compounds, and _Leptospirillum ferrooxidans_, which can get its energy only by oxidizing Fe^{2+} but not reduced sulfur compounds. Growth of the thermophiles is stimulated or is dependent upon a trace of yeast extract in laboratory culture media. The reason for this is not presently understood.

Ferrous iron, reduced sulfur compounds, metal sulfides, uranium (IV) compounds, or selenide can serve as energy sources to the acidophilic chemolithorophs by virtue of the fact that electrons removed in the oxidation process are fed into the electron transport system (ETS) of the bacteria and transmitted to the terminal electron acceptor (usually oxygen)(Fig. 1). The passage of electrons down the ETS to oxygen establishes an electrochemical gradient across the plasma membrane and the protons (H^+) generated in the oxidation of the metal compound generate a pH gradient across this membrane. The sum of the two gradients, expressed in volts, constitutes the proton motive force which the cell uses to generate high-energy phosphate bonds by combining ADP and P_i to form ATP with the help of the membrane-bound enzyme ATPase. The high-energy phosphate bonds store energy which a cell uses to drive energy-consuming metabolic reactions.

Not all electrons from the energy-source oxidation are passed to oxygen. In growing cells, a significant portion of the electrons are passed by the ETS to phosphopyridine nucleotides (see Fig. 1). Since electrons pass against the redox gradient to $NADP^+$, the cell has to expend energy to get electrons to $NADP^+$.

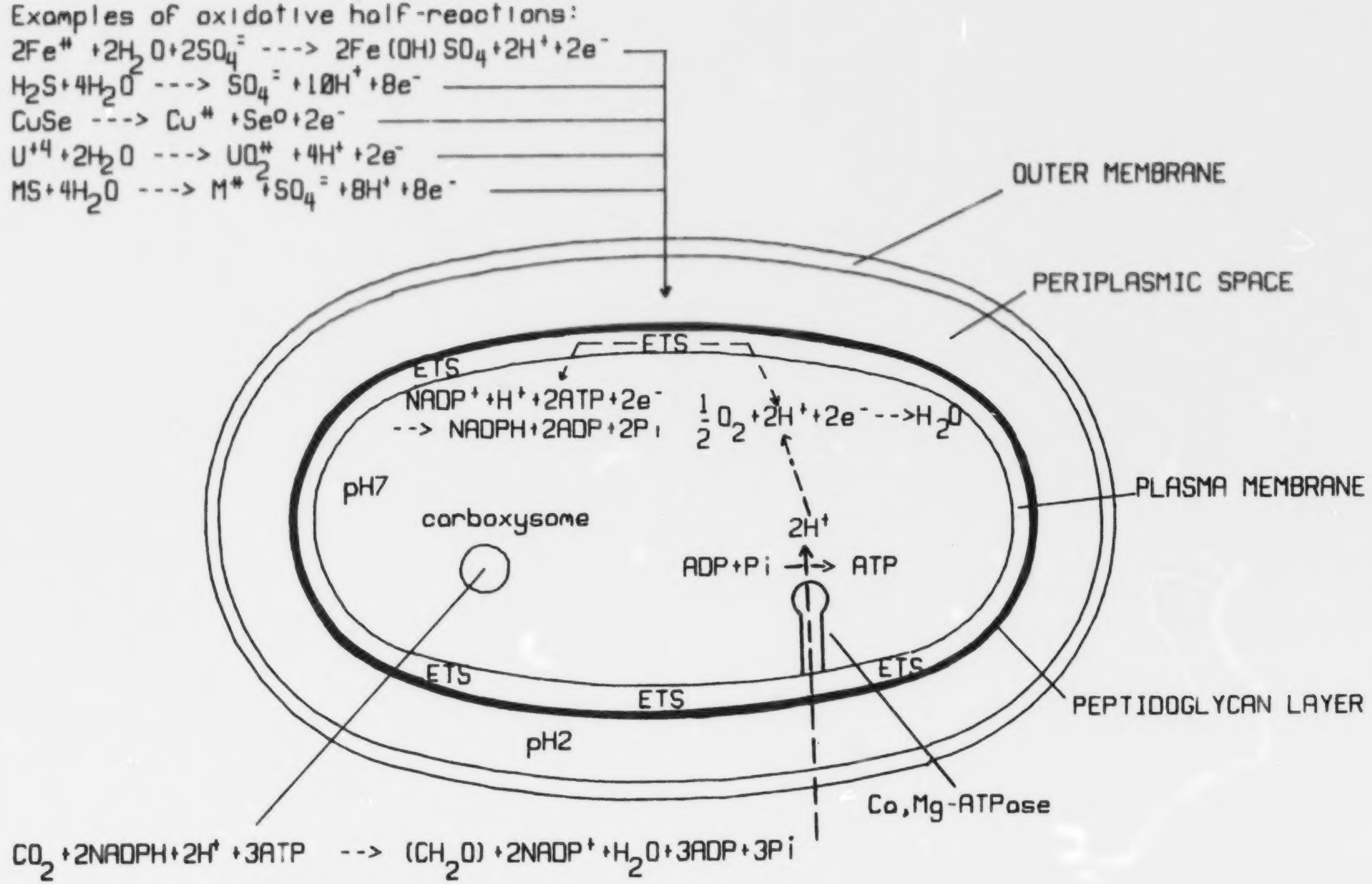

Examples of oxidative half-reactions:
2Fe## +2H2O+2SO4= ---> 2Fe(OH)SO4+2H++2e-
H2S+4H2O ---> SO4= +10H+ +8e-
CuSe ---> Cu## +Se0+2e-
U+4 +2H2O ---> UO2## +4H+ +2e-
MS+4H2O ---> M## +SO4= +8H+ +8e-
OUTER MEMBRANE
PERIPLASMIC SPACE
ETS
ETS
NADP+ +H+ +2ATP+2e- --> NADPH+2ADP+2Pi
1/2 O2 +2H+ +2e- -->H2O
PLASMA MEMBRANE
pH7
carboxysome
2H+
ADP+Pi --> ATP
ETS
ETS
ETS
PEPTIDOGLYCAN LAYER
pH2
Co,Mg-ATPase
CO2 +2NADPH+2H+ +3ATP --> (CH2O) +2NADP+ +H2O+3ADP+3Pi

Figure 1 Model of a cell of Thiobacillus ferrooxidans showing sites where key biochemical reaction occur during oxidation of metal compounds. See text and glossary for identification of terms.

This energy is obtained by hydrolysis of ATP to ADP + P_i. A minimum of 2 ATP's are needed to reduce one $NADP^+$.

NADPH + H^+ is needed in the assimilation of CO_2, the carbon source of chemolithotrophs. After carboxylation of an organic receptor molecule (e.g., ribulose bisphosphate), the resultant product (phosphoglyceric acid) is reduced by NADPH + H^+. This reduction consumes energy in the form of ATP. The reduced product then is the point of departure for the synthesis of the various organic protoplasmic constituents of the cell as well as for fresh ribulose bisphosphate (Calvin cycle) for additional CO_2 fixation and reduction. The synthesis of organic carbon from CO_2 can be summarized by

$$[CO_2] + 2NADPH + 2H^+ + \text{energy} = [CH_2O] + H_2O + 2NADP^+.$$

The amount of energy released in the oxidation of one inorganic molecule such as Fe^{2+}, H_2S, S^o, U(IV), etc. is small (Table 5). Since the energy demand of the cell is large (the assimialtion of 1 mole of CO_2 can be estimated to require about 118 kcal of energy (Thauer et al., 1977; Sokatch, 1969), it can be seen that a lot of an inorganic energy source has to be oxidized to meet this energy demand. It is for this reason that the chemolithotrophic bacteria which can attack FeII, reduced sulfur compounds, metal sulfides, U(IV), CuSe, etc. are so useful in leaching. They must oxidize so much of their energy source to be able to grow.

The enzyme systems involved in the oxidation of the inorganic energy sources are in many cases only partially understood. The currently best understood system is the iron oxidase system in T. ferrooxidans. The enzyme which oxidizes ferrous to ferric iron has been isolated from the periplasmic space of T. ferrooxidans cells (Cox and Boxer, 1978). It was found to be a copper enzyme called rusticyanin, which transfers electrons from iron to c-type cytochrome, which in turn passes

Table 5. Standard free energies for some biohydrometallurgically important reactions

$Fe^{2+} + \frac{1}{4}O_2 + H^+ \dashrightarrow Fe^{3+} + \frac{1}{2}H_2O$	G_r = -10.57 kcal/mol
$H_2S + \frac{1}{2}O_2 \dashrightarrow S^o + H_2O$	G_r = -50.14 kcal/mol
$H_2 + 2O_2 \dashrightarrow SO_4^{2-} + 2H^+$	G_r =-170.8 kcal/mol
$S^o + 3/2\ O_2 + H_2O \dashrightarrow 2H^+ + SO_4^{2-}$	G_r =-120.65 kcal/mol
$CuSe + \frac{1}{2}O_2 + 2H^+ \dashrightarrow Se^o + H_2O + Cu^{2+}$	G_r = -33.26 kcal/mol
$U^{4+} + \frac{1}{2}O_2 + H_2O \dashrightarrow UO_2^{2+} + 2H^+$	G_r = -42.51 kcal/mol

(References: Ehrlich, 1978; Garrels and Christ, 1965) the electrons to cytochrome a on the inside of the the plasma membrane, which then reduces oxygen to water in the cytosol (Fig. 2)(Cobley and Haddock, 1975; Ingledew et al., 1977). The enzyme involved in the oxidation of S^o is an oxygenase which causes the formation of sulfite in air. Glutathione appears to be a needed cofactor (Suzuki and Silver, 1966). Sulfite is then oxidized by oxygen to sulfate by one of two possible pathways, one requiring the participation of adenylic acid (AMP) and the other not. Only the sulfite-oxidizing systems yield useful energy, 1 1/2 or 1 ATP per sulfite molecule oxidized, respectively (see Ehrlich, 1981). The enzyme for oxidizing H_2S is poorly characterized, as are the enzymes for U(IV) oxidation, CuSe oxidation, etc.

3.1.2.2 Regulation of Metabolism

The energy metabolism and the assimilatory metabolism of any cell consists of many interdependent and interconnected biochemical reaction sequences. Not all of these sequences need to operate at optimum speed at all times. Otherwise the metabolic system may become unbalanced leading to overproduction or underproduction of a vital product. To keep the metabolism of a cell in balance, nature has invented various regulatory mechanisms.

For instance, in prokaryotes like bacteria, some enzymes in the cell are always present regardless of growth conditions (constitutive enzymes), whereas others are only formed when needed (inducible enzymes). By the same token, some membrane transport proteins (permeases) may also be present only when needed. The formation of some enzymes may be suppressed under special growth conditions (enzyme repression). Such repression may result because of direct action of specific substrates, or it may be because a substrate causes the destruction of cyclic adenylic acid (cAMP) which is needed in promoting the formation of a particular enzyme or series of enzymes. The protein of an

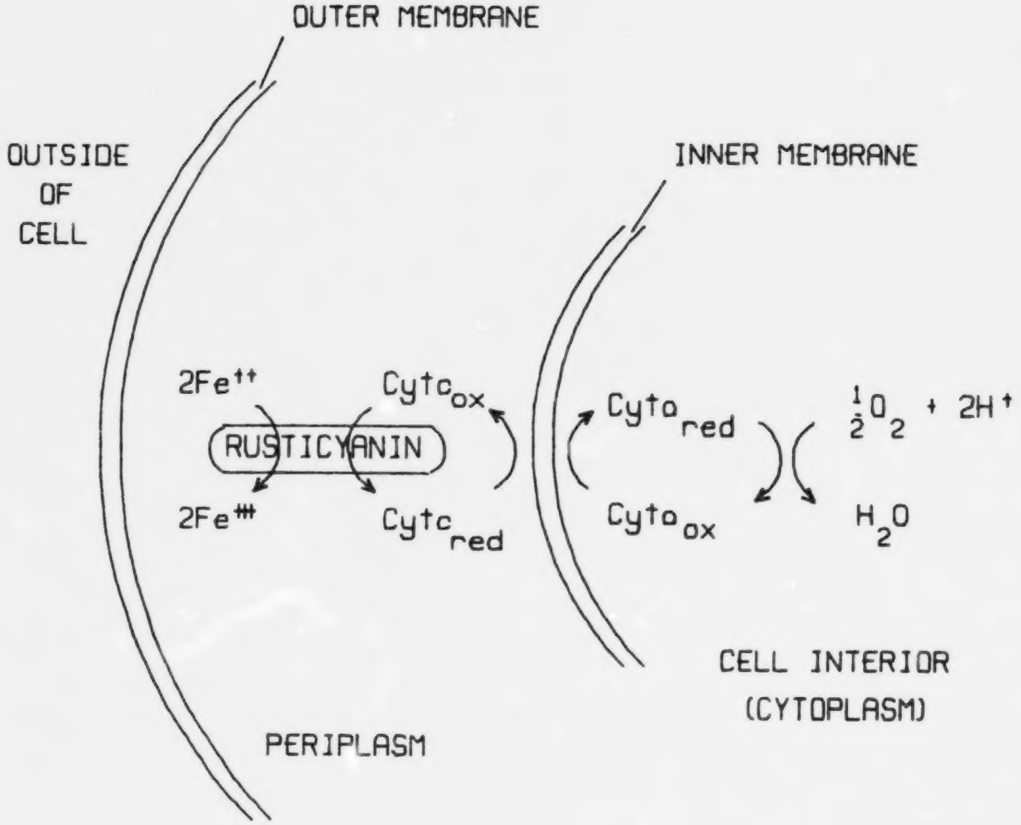

OUTER MEMBRANE
OUTSIDE OF CELL
INNER MEMBRANE
2Fe++
RUSTICYANIN
2Fe+++
Cytc ox
Cytc red
Cyta red
Cyta ox
1/2 O2 + 2H+
H2O
CELL INTERIOR (CYTOPLASM)
PERIPLASM

Figure 2 Model of Fe^{++} oxidation by Thiobacillus ferrooxidans

enzyme in some metabolic sequences, especially ones leading to synthesis, may exhibit different conformations (allosterism), depending on the concentration of the product formed by the enzyme sequence. In excess, some of the product (effector) may bind to a specific site on the allosteric enzyme (feedback inhibition) and weaken its catalytic activity. Once the concentration of the effector has dropped below a critical level in the cell, the effector on the allosteric enzyme dissociates from it and the enzyme assumes its original, more active configuration, allowing for resumption of product formation. Variation in pH and/or Eh can also regulate enzyme activity because each enzyme has an optimum pH and Eh at which it works best, and outside of which it is less active.

The activity of bacteria may be modulated by modifying or eliminating some of the regulatory mechanisms. For instance, inducible enzymes can be made constitutive by eliminating the control mechanism. Similarly, the mechanism for enzyme repression may be eliminated so that it is formed even under unfavorable conditions. The sensitivity to catabolite repression of enzyme synthesis may be eliminated and allosterism of enzyme proteins may be modified or eliminated. All these modifications require genetic alteration, because it is specific genetic information in the DNA of a cell which determines these regulatory mechanisms and their mode of operation.

Modification or elimination of one or more metabolic regulating mechanism may be manifested in faster or slower growth rates, or more or less active metabolic activity. This can have an obvious bearing on the leaching activity of bacteria or their propensity for bioaccumulation of metals.

3.1.2.3 Role of Inheritance and Environmental Factors

The structural organization and the physiological capability of any cell are genetically determined. By this is meant that a cell has encoded in its genetic repository (DNA) all the information necessary for the operation of the biochemical processes needed for cell construction and operation, i.e., for cell replication, cell motility, cell differentiation, etc. Each of a series of segments on the DNA polymer (structural genes) specify the amino acid sequence of a different protein or protein subunit whose function may be enzymatic or that of regulation of protein synthesis. Some other segments of the DNA of a cell (regulatory genes) may play a regulatory function in reading out of the structural genes. They do not specify proteins. Structural genes must first be transcribed into messenger ribonucleic acid (m-RNA). This RNA is translated into a peptide chain (a heteropolymer of amino acids) that folds into a specific configuration predetermined by its amino acid sequence and constitutes part or all of a particular protein. Some proteins are intimate associations of more than one folded polypeptide chain, which may all be of the same kind or of two or more different kinds. The translation of messenger RNA into peptide takes place on a ribosome and involves the sequential linking of amino acids specified by the m-RNA with the formation of peptide bonds between them. The amino acids are carried to the ribosome on special transfer RNA (t-RNA). It is t-RNA's which recognize the amino acid sequence specified by the m-RNA and enable the system to assemble the amino acids in the proper order.

Although the basic principles of how genetic information is utilized is the same in prokaryotes and eukarykotes, the details of the mechanism differ. The extent of these differences have still not been fully unraveled. In eukaryotic cells, m-RNA is synthesized in the nucleus of the cell because that is where the chromosomes, which carry the genetic information (DNA), are located. Much of the m-RNA produced in the nucleus has to be further processed before it can be exported into the cytoplasm of the cell and be translated into peptide or protein on appropriate ribosomes. Some protein synthesis occurs within organelles such as mitochondria and chloroplasts of eukaryotic cells. These particular syntheses follow mechanisms more like those of prokaryotes.

The expression of some genes is under environmental influence as discussed in section 3.1.2.2. Thus genes which code for inducible enzymes are expressed only when the substrate for the enzyme or a structurally similar compound is present in the environment around the cell and no other environmental factor prevents the expression of such a gene by, for instance, catabolite repression. The formation of some enzymes may be repressed by a specific environmental factor to prevent overproduction of the enzyme product.

Genes may be modified (mutated) by specific chemical or physical factors (mutagens). Examples of chemical factors are nitrogen mustard, nitrous acid, and bromouracil. Examples of physical factors are ultraviolet- and X-radiation and heat. If such a modification affects a structural gene, the result may be an altered gene product (protein) with attenuated or enhanced activity, or it may no longer code for a gene product (lethal mutation if the protein is vital to the cell). If the gene is not a structural gene but has a regulatory function over structural gene expression (enzyme induction or repression), its mutation may lead to loss of the control function. In this way, an inducible enzyme may be made into a constitutive one. Since genes also control the permeability of cells to specific solutes by coding for specific membrane proteins involved in transport of solutes through the membrane, and since the expression of these genes may be modified by environmental factors (mutagens), cell permeability may be environmentally controlled like enzyme synthesis.

Genes can undergo "spontaneous" mutation, i.e., they can become modified due to an error in replication, or due to an encounter with a naturally occurring mutagenic agent (chemical or physical). Such spontaneous mutations are very rare events, in bacteria they may occur in the order of 1 in 10^7 cells in a population. A mutation may be lethal if a vital gene has been destroyed, or it may result in an altered genetic trait if the modified gene can still express itself. In a microbial population, the wild type (unmutated parent) cells will ordinarily outnumber mutants by many orders of magnitude. However, if the environment is modified to favor the growth of the mutant, the mutant may eventually outgrow the wild type after many growth cycles. For instance, if a spontaneous mutant in a wild type population is Cu^{2+} resistant whereas the wild type parent is not, incorporating Cu^{2+} into the medium at levels toxic to the wild type but not the mutant, will cause the mutant to overgrow the wild type after many growth cycles.

Single or multiple genes can be transferred unidirectionally from a donor- to a recipient prokaryote with the possibility of gene recombination. Mechanisms of gene tranfer include (1) transformation, (2) transduction, and (3) conjugation (Fig. 3). In transformation, a short piece of DNA carrying 1 or at most 2 or 3 closely linked genes from a lysed (disintegrated) donor cell may be picked up by a competent recipient cell and be incorporated in its chromosome. If the acquired gene(s) express a trait not previously possessed by the recipient, it was genetically modified in respect to these traits. In transduction, a short piece of donor-cell DNA, equivalent to 1 or at most 2 or 3 closely linked genes, is transferred to a recipient cell by a bacteriophage (bacterial virus) after lysis of the donor cell in which the bacteriophage picked up the DNA. The bacteriophage infects the recipient cell without multiplying in it. Instead the piece of DNA it carried may be integrated into the chromosome of the recipient as in transformation. In conjugation, a physical connection is formed between donor and recipient cell such that donor DNA can be transferred to the recipient cell via the cell connection (e.g., sex pilus) and be

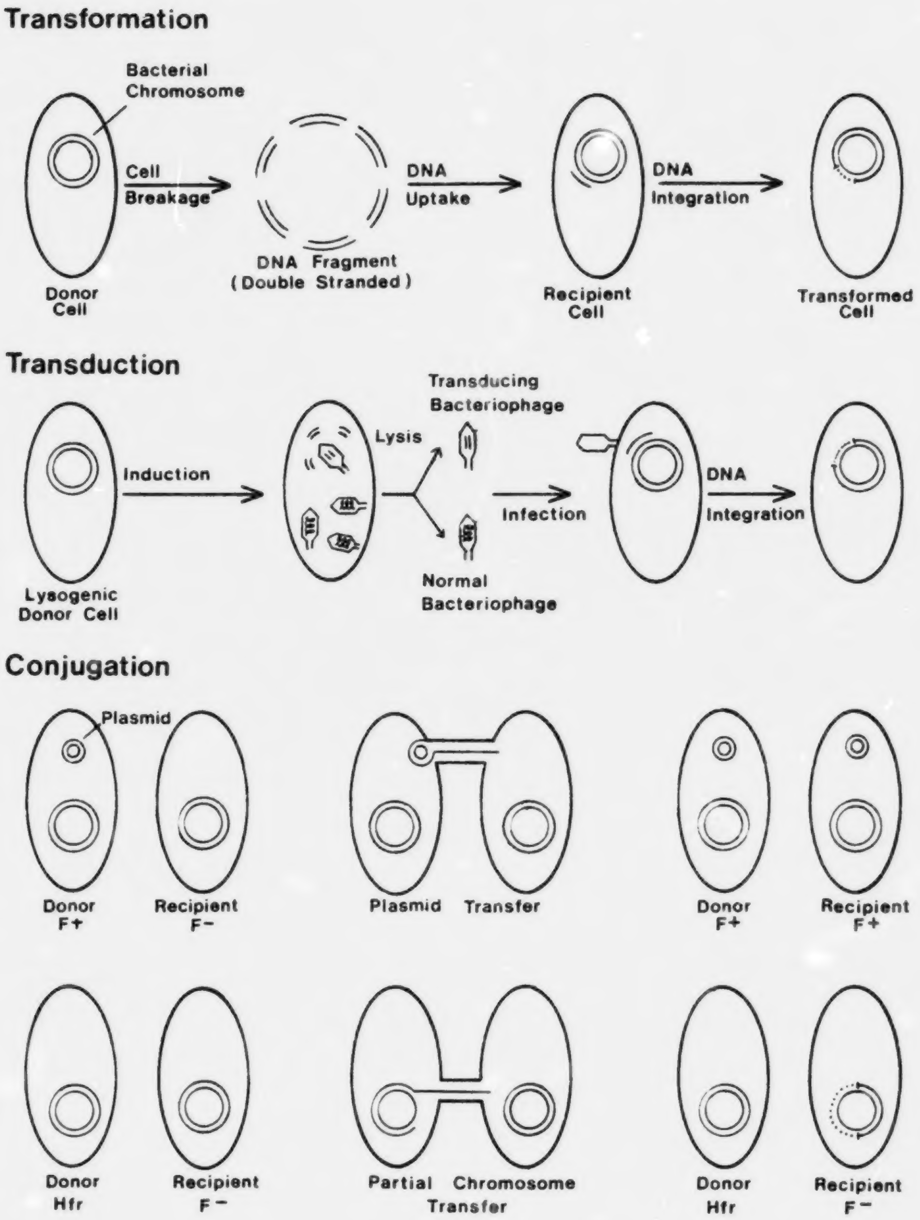

Transformation
Bacterial Chromosome
Cell Breakage
DNA Uptake
DNA Integration
DNA Fragment (Double Stranded)
Donor Cell
Recipient Cell
Transformed Cell
Transduction
Transducing Bacteriophage
Lysis
Induction
Infection
DNA Integration
Lysogenic Donor Cell
Normal Bacteriophage
Conjugation
Plasmid
Donor F+
Recipient F−
Plasmid Transfer
Donor F+
Recipient F+
Donor Hfr
Recipient F−
Partial Chromosome Transfer
Donor Hfr
Recipient F−

FIGURE 3: THREE MECHANISMS OF GENE (DNA) TRANSFER IN BACTERIA

integrated into the DNA of the recipient cell. The DNA which is transferred may be chromosomal or extra-chromosomal (plasmid) DNA. Plasmid DNA is generally more readily transferred than chromosomal DNA. Also, plasmid DNA transfer does not require the interacting cells to be of the same species or even genus. The total amount of DNA transferred can be much greater than in either transformation or in transduction, i.e., many more than 1, 2, or 3 genes can be transferred in one conjugational event. When several genes are transferred, they pass from donor to recipient in a specific sequence because genetic loci are linearly arranged in the threat-like DNA molecule (see Fig. 4).

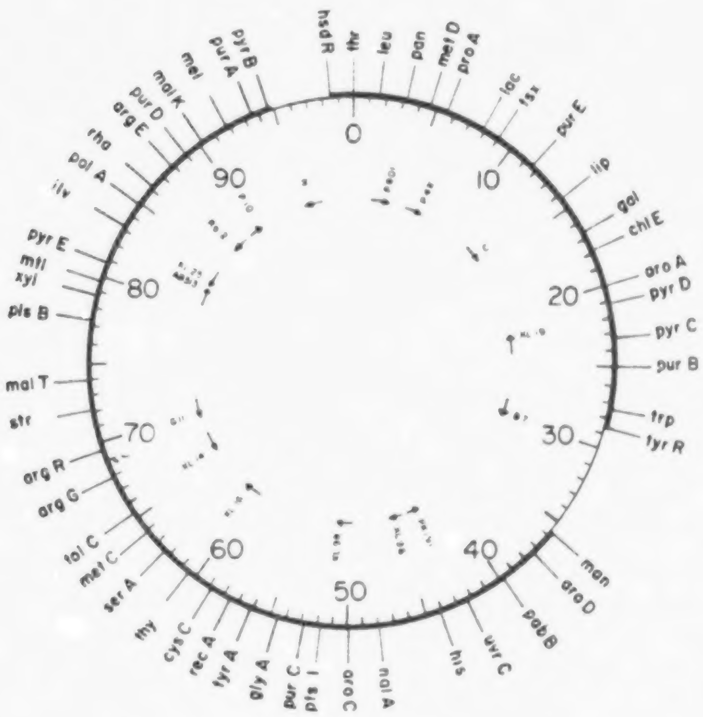
In eukaryotic cells, genes may be exchanged between homologous chromosomes, usually during reduction division (halving of the chromosome number associated with sexual reproduction), more rarely during chromosomal duplication (associated with non-sexual reproduction). This also leads to gene recombination. Genes on any eukaryotic chromosome are linearly arranged, and for this reason closely linked genes in eukaryotes are less frequently segregated than distantly linked genes on a chromosome.

3.1.2.4. Mutualistic Associations

Some microorganisms grow better in mixed culture with one or more taxonomically unrelated microorganisms (consortium). In a sense, these microorganisms complement each other metabolically. Mixed cultures may be more effective in leaching ores than pure cultures (Lundgren and Malouf,1983).

Many strains of Thiobacillus ferrooxidans have been found to be mixed cultures of the Thiobacillus and another acidophilic bacterium, such as Thiobacillus acidophilus, Acidiphilum cryptum, or others (Guay and Silver, 1975; Harrison, 1981; Zavarzin, 1972), which cannot grow on FeII. The non-iron oxidizers consume

FIGURE 4. Circular reference map of E. coli K-12. The large numbers refer to map position in minutes, relative to the thr locus. From the complete linkage map, 52 loci were chosen on the basis of greatest accuracy of map location, utility in further mapping studies, and/or familiarity as long-standing landmarks of the E. coli K-12 genetic map. The two thin portions of the circle represent the only two map intervals that are not spanned by a continuous series of P1 cotransduction linkages. Inside the circle, the leading transfer regions of a number of Hfr strains are indicated.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hsd R
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pro A
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tsx
pur E
lip
gal
chl E
aro A
pyr D
pyr C
pur B
trp
tyr R
man
aro D
pab B
uvr C
his
nal A
aro C
pls I
pur C
gly A
tyr A
rec A
cys C
thy
ser A
met C
tol C
arg G
arg R
str
mal T
pls B
xyl
mtl
pyr E
ilv
pol A
rha
arg E
pur D
mal K
mel
pur A
pyr B

Source: B. J. Bachman, K. B. Low, and A. L. Taylor, Bacteriological Reviews, 40, 118 (1976). Reproduced with permission of the American Society for Microbiology.

organic excretions such as pyruvate and amino acids by T. ferrooxidans as energy and carbon sources. The organic excretions have a growth-inhibitory effect on T. ferrooxidans, and their removal facilitates the growth of the bacterium (Arkesteyn and De Bont, 1980).

In the laboratory, T. ferrooxidans has benefitted from culture with Beijerinckia lacticogenes (Tsuchiya, 1978), a nitrogen-fixing bacterium, when nitrogen in the medium was growth limiting. Tuovinen et al. (1979) could not confirm these findings. However, it is now known that some strains of T. ferrooxidans can fix their own nitrogen (Macintosh, 1978). Thus Tuovinen et al. (1979) may have used a strain of T. ferrooxidans capable of nitrogen fixation.

Both T. ferrooxidans and T. thiooxidans can oxidize reduced forms of sulfur while T. thiooxidans cannot, however, oxidize iron. Some leaching processes seem to benefit from the presence of both of these organisms (Norris and Kelly, 1978). The reason for this is not entirely clear.

In acid mine drainage, a mixture of thiobacilli, heterotrophic bacteria, mycelial fungi and yeasts, and protozoa are usually found (see, for instance, Ehrlich, 1981). These organisms are very acid tolerant and may also be very metal tolerant. Their coexistence in acid mine drainage indicates a nutritional interdependency (food web), but the full nature of this interdependency needs to be unraveled.

3.1.2.5 Adaptation by Subculturing

In the literature, several reports exist which indicate that metal-tolerance of Thiobacillus ferrooxidans strains can be increased by serial subculture in the presence of increasing amounts of a toxic metal (Zimmerley et al., 1958). This adaptation has been called training. Exactly what is involved in this training has not been worked out by these investigators. It may be a matter of selecting spontaneous mutants through serial subculture (Grudeva and Grudev, 1980). Or it could involve the spread and/or amplification of special plasmids in the bacterial population.

3.1.2.6 Genetic Manipulation Experiments

As suggested by the genetic behavior of microbes described in section 3.1.2.3, microbes may be genetically altered by adapting naturally occurring, gene modifiying processes to laboratory manipuilation. Mutation rates may be enhanced by applying higher doses of mutagens than are likely to be encountered in nature. Gene transfer can be enhanced by optimizing conditions for transformation, transduction, or conjugation in the case of prokarykotes, or by enhancing sexual reproduction in the case of eukarykotes.

Recently it has become possible to modify DNA in the laboratory in ways that are unlikely to occur in nature and thus to have cells which express traits that they would not acquire in their normal life (genetic engineering). In the case of bacteria, such modifications involve cleaving of native, circular DNA (frequently plasmid DNA) with restriction enzyme, inserting a piece of foreign DNA encoding one or more genetic loci from another, often quite unrelated cell, with the help of DNA ligase, and then implanting the modified plasmid into a bacterial cell by a process like transformation. Instead of adding genetic information to a plasmid, genetic information can also be deleted by excising it with restriction enzymes and then recircularizing the shortened plasmid DNA with DNA ligase and implanting it into a recipient bacterium. Bacterial plasmids may carry genes for certain metabolic functions, for drug resistance, and for metal ion resistance. There are different kinds of plasmids, and not all can coexist in the same cell, i.e., they may be incompatible with each other. Some plasmids can combine in a cell and form a larger plasmid. If the plasmids are all of the same kind, the resultant plasmid is a multimer. Some plasmids can acquire genes from the chromosome of their host cell. Others may transfer some or all of their genes to the host chromosome via transposons. These properties of plasmids can be utilized in genetic engineering.

Genetic engineering of metal-solubilizing and metal-concentrating bacteria is in its infancy. This is because the genetic properties of organisms like Thiobacillus ferrooxidans, T. thiooxidans, Sulfolobus spp., Leptospirillum ferrooxidans, thermophilic thiobacilli, and others have not been clearly worked out. Plasmids have been found in T. ferrooxidans (Mao et al., 1980; Martin et al., 1981), but what they control remains to be discovered. In some cases it is suspected but not proven that they may confer resistance to uranium (Martin et al., 1981).

Manganese-oxidizing capacity seems to be plasmid-borne in some bacteria (Gregory and Staley, 1982; and Engebrecht et al., 1982). The genetic locus for chromate reduction in a strain of Pseudomonas fluorescens, on the other hand, resides on the bacterial chromosome, even though chromate resistance in this organism is determined by a plasmid (Bopp, 1980). Hg^{2+} reducing capacity in bacteria is generally plasmid- determined (Summers and Silver, 1978).

The plasmid-determined activities cited above and others (Table 6) can be subjected to manipulation by genetic engineering and be thereby introduced into organisms previously incapable of particular activities on metals determined by these plasmids. The organisms listed in Table 6 and others not listed represent a reservoir of plasmids that genetic engineers can draw upon for introduction into bacterial strains to give them new desirable properties for use in metal extraction or concentration.

Table 6. Microbial reaction with metals which are genetically determined on plasmids

Metal	Organism(s)	Determinant function	Reference
Ag	*Staphylococcus aureus*	Resistance	Summers et al., 1975
As	*E. coli*; *Staph. aureus*	Resistance	Summers & Silver, 1978
Cd	*Staph. aureus*	Resistance	Summers & Silver, 1978
Cr	*Pseudomonas fluorescens*	Resistance	Bopp et al., 1983
Cu	*Escherichia coli*	Resistance	Tetaz and Luke, 1983
Hg	*Pseudomonas aeruginosa*, *E. coli*, *Staph. aureus*	Resistance and reduction	Summers & Silver, 1978
Mn	Unnamed bacteria	Manganese oxidase	Gregory & Staley, 1982; Engebrecht et al., 1982
Pb	*Staph. aureus*	Resistance	Summers & Silver, 1978
Sb	*Staph. aureus*	Resistance	Summers & Silver, 1978
Te	*Pseudomonas* sp.	Resistance	Summers & Silver, 1978

3.1.3 Mechanistic Studies

3.1.3.1 Direct and Indirect Action

In bioleaching of ores, the action of the microorganisms may be direct, indirect, or a combination of both actions. Direct action involves direct enzymatic attack of the crystal lattice by the microorganisms and involves either an oxidation or a reduction of a lattice component. For instance, in direct attack of covellite (CuS) by T. ferrooxidans, the sulfide is oxidized to sulfate via elemental sulfur (Ehrlich, 1978, 1981; Silverman and Ehrlich, 1964). In order to be able to attack the crystal lattice, bacteria must be in intimate contact with the crystal surface. Indeed, bacteria such as T. ferrooxidans have been shown attached to crystal surfaces during active leaching (Tributsch, 1976; Bennett and Tributsch, 1978; Imai, 1978). However, it remains unclear how the bacteria can actually attack an atom in a crystal lattice. One prevailing idea is that they are able to abstract electrons in cathodic areas (e.g., sulfide regions) and thereby cause release of cations in anodic regions (e.g., Cu^{2+}). Another idea is that the bacteria form a chelating agent which causes local solubilization of a crystal by complexing, for instance, the metallic cations and enables the bacteria to oxidize the solubilized reduced species (Tributsch, 1976; Bennett and Tributsch, 1978). No evidence exists currently that T. ferrooxidans produces such a chelating agent. Another idea is that protons and Fe^{3+} generated by T. ferrooxidans act as catalytic agents in the oxidation of metal sulfides (Tributsch and Bennett, 1981a, b). These protons or ferric ions are seen as reacting with metal sulfide surfaces "by breaking chemical bonds and shifting electron states", the proton or ferric ion being recycled in this process. Of these proposed mechanisms, the first is the most favored at present.

Indirect action involves the generation of a metabolic product by a microorganism which reacts chemically with an ore and/or gangue mineral causing its solubilization. Such metabolic products may be oxidizing or reducing agents, chelating agents, or organic or inorganic acids. In the bioleaching of metal sulfides, the metabolic product may be ferric sulfate in acid solution formed by T. ferrooxidans or other iron oxidizer from ferrous iron. The ferric iron then attacks the metal sulfide (MS),

$$MS + 2Fe^{3+} = M^{2+} + S^{o} + 2Fe^{2+}.$$

A specific example is the bioleaching of uranium by the indirect method,

$$UO_2 + 2Fe^{3+} = UO_2^{2+} + 2Fe^{2+}$$

T. ferrooxidans and/or other acidoplhilic iron oxidizers then reoxidize the ferrous iron back to ferric iron (see Ehrlich, 1981).

In the leaching of manganese from pyrolusite, it may be oxalate formed by fungi from glucose which reduces the MnO_2 to Mn^{2+},

$$MnO_2 + 2HOOCCOOH + 2H^{+} = Mn^{2+} + CO_2 + 2H_2O.$$

In the solubilizing of Fe_2O_3, it may be a siderophore which is a very strong chelator of ferric iron but not ferrous iron (see section 3.1.3.2; also Lankford,1973), which solubilizes ferric iron.

In the leaching of chromite ore, it may be H_2SO_4 generated from elemental sulfur by T. thiooxidans which extracts some Cr and Fe (Ehrlich, 1983b).

Rarely does a bioleaching process involve just one direct or indirect mechanism. In most instances, both types are involved. That is particularly true in the leaching of metal sulfides (see discussion by Ehrlich, 1981). It is often difficult to assess accurately by experiment how much each biological mechanism contributes to leaching. It is less difficult to assess how much biological leaching contributes in addition to chemical leaching, if it is possible to include a poison control in the experimental assessment or if a sterile control is run concurrently.

3.1.3.2 Role of Bioligands and Biosurfactants

Bioligands such as the siderophores, oxalic, citric, and 2-ketogluconic acids may play very important roles in mineral solubilization. Siderophores, whose binding constant for ferric ion ranges from 10^{38} to 10^{50} as compared to their binding constant for ferrous ion which is around 10^{8}, are very important biologically in solubilizing ferric iron as it occurs in Fe_2O_3, FeOOH, and $Fe(OH)_3$. Siderophores are hydroxamate or catechol derivatives or exhibit a mixture of these traits (Neilands, 1973). Citric acid may also act as a siderophore (e.g., Messenger and Ratledge, 1982). However, citric, oxalic and 2-ketogluconic acids also chelate alkaline earths and other metallic cations in silicates and aluminosilicates, causing their slow solution (see discussion by Ehrlich, 1981; Silverman and Munoz, 1970, 1971). Fulvic acid components of humus can chelate metals (Schnitzer, 1971) as can certain lichen acids (Schatz, 1962).

Biosurfactants play an important role in the oxidation of elemental sulfur by *Thiobacillus thiooxidans*. They are produced by the *Thiobacillus* to wet the sulfur in preparation for its attack by the organism (Jones and Starkey, 1961; Schaeffer and Umbreit, 1963; Cook, 1964). The chemical nature of these surfactants is mainly that of phosphatidyl N-methylethanolamine, phosphatidyl glycerol and diphosphatidylglycerol (Jones and Benson, 1965; Shively and Benson, 1967).

Surfactants have also been claimed to facilitate leaching of metal sulfides such as chalcopyrite (Ducan et al., 1964; Duncan and Teather, 1966). This claim has not, however, been substantiated in later experimentation (see Torma, 1977). Indeed, surfactants seem to be inhibitory to bioleaching of sulfide ores.

3.1.4 Effects of Mineralogy on Leaching

3.1.4.1 Resistance of Ores to Leaching

Although the generalization can be made that specific types of ores and ore concentrates, e.g., metal sulfide-containg ores, can be bioleached, experience has shown that not every ore of a partricular type actually responds to such treatment. The reasons for this vary. They include

1. too much of an acid-consuming component like $CaCO_3$ in the gangue, in the case of metal-sulfide ore leaching by T. ferrooxidans and/or other acidophilic bacteria. In some instances, an acid wash may remove enough of the acid-consuming component to make subsequent bioleaching by an acidophile(s) possible. In some instances the biooxidation of an ore component such as chalcocite (Cu_2S) consumes enough acid which if not replaced will bring the leach process to a halt.

$$Cu_2S + 5/2\,O_2 + 2H^+ = 2Cu^{2+} + SO_4^{2-} + H_2O.$$

If pyrite occurs in the ore, its biooxidation will generate enough excess acid to overcome the acid consumption by chalcocite.

2. Presence of one or more toxic mineral components which when solubilized inhibit the biological leaching agent. For example, T. ferrooxidans but not Sulfolobus spp. is inhibited by molybdate ion, which is formed in the oxidation of molybdenite. Molybdate toxicity can, however, be overcome if the bioleaching process generates ferric iron which forms ferric molybdate and thus lowers its toxicity for T. ferrooxidans. Other ore constituents which are potentially troublesome include mercury- containing and silver-containing minerals.

3. The gangue of a metal sulfide ore may have a significant adsorption capacity for leached metal ions, such as cupric ions, and thus diminish the recovery of the metal ions in the pregnant solution (Ehrlich, 1977). This problem can be at least in part overcome by proper pH control of the leaching process, because the metal ion adsorption is pH dependent. Final acid washing of the leached ore may also help to recover adsorbed metal ions.

4. Galena (PbS) presents a special problem in bioleaching with T. ferrooxidans because the product of leaching ($PbSO_4$) is a relatively insoluble compound. It tends to form on the mineral surface and passivate it, i.e., preventing further oxidation of the galena by the bacteria. Silver and Torma (1974) found that they could prevent the passivating layer of lead sulfate from forming by continual agitation (shaking) of the reaction mixture.

5. The mineral component of an ore may be so finely disseminate in the gangue (locked in) that it is not readily accessible to the leaching microbes or the lixiviant they generate unless they or their lixiviant can also dissolve away the gangue from around the ore mineral. A possible example of this is the leaching of Shebandowan chromite ore with dilute sulfuric acid generated by T. thiooxidans from sulfur (Ehrlich, 1983b).

6. Although this is not likely to be a significant problem with

bulk ore or ore concentrate, bacteria like T. ferrooxidans was attacking the crystal lattice of a mineral directly seem to require imperfections in the lattice to initiate the attack (Silverman et al., 1961; Bennett and Tributsch, 1978). Perfect crystals seem to resist their attack.

3.1.4.2 Secondary Mineral Formation

In bioleaching inorganic products with low solubility may be formed which may be detrimental to the progress of the leach process. One example has already been cited (section 3.1.4.1) in regard to bioleaching of galena, in which the insoluble lead sulfate which is formed may passivate the crystal surfaces of the remaining galena and prevent its further oxidation.

A more general problem in bioleaching of metal sulfide is the formation of jarosite (Ivarson, 1973; Ivarson et al., 1979; Tuovinen and Carlson, 1979), a very insoluble basic ferric sulfate. Since iron sulfides (e.g., pyrite (FeS_2), chalcopyrite ($CuFeS_2$), bornite (Cu_5FeS_4), etc.) are generally always present in varying amounts in sulfide ore bodies, the formation of jarosite in leaching will inevitably occur. This compound will precipitate on unleached sulfide mineral and passivate it. In leach dumps and in in-place leaching of ore bodies it will precipitate in the flow channels through which the leach and pregnant solutions must pass, causing obstruction and thus interfering with flow (LeRoux and Mehta, 1978). This problem can be minimized by controlling the ferric iron concentration in the leach dump or ore body that is being leached in-situ through control of the ferric iron level in the leach solution (Zimmerley et al., 1958), and in the case of leach dumps, bulldozing the tops of the dumps. The concentration of ferric iron in the leach solution can be controlled in oxidation ponds if the source of the ferric iron is the ferrous iron of barren solution (Zimmerley et al., 1958; see also Ehrlich, 1981). In these ponds, T.

ferrooxidans is allowed to oxidize ferrous iron and to cause partial precipitation of the ferric iron as jarosite.

When senarmontite (Sb_2O_3) or servantite (Sb_2O_4) are oxidized to antimony pentoxide (Sb_2O_5) by Stibiobacter senarmontii, the ultimate product is stibiconite [$Sb_3O_6(OH)$], a solid (Lyalikova, 1974), which could also cause problems in continuous leaching.

When sulfuric acid is generated by bacteria from sulfide minerals or from sulfur in the presence of $CaCO_3$ or igneous minerals like Ca-containing feldspar, gypsum ($CaSO_4.2H_2O$) may be formed as a secondary mineral (Ivanov, 1968).

The attack of igneous aluminosilicate or silicate minerals by acid may lead to their transformation into clay minerals (Ivarson et al., 1978), for instance $2KAlSi_3O_8 + H_2CO_3 + H_2O = H_4AlSi_2O_9 + K_2CO_3 + 4SiO_2$.

3.1.4.3 Mineral Feed Stock Variations

In the bioleaching of metal sulfides, the feed stock may be

1. low-grade ore varying in particle-size from boulders to small particulates of 1 mm or less in diameter, representing a fractured ore body or ore waste in a leach heap or dump;

2. ground, but not beneficiated ore of graded size;

3. ore concentrate of graded size (see below);

4. tailings from an ore processing plant (Fabiszewska et al., 1978).

5. smelter slag.

The ore may contain predominantly one metal value or a mixture of metal values, or it may contain the same kind of metal value but in the form of several different minerals.

The leaching of ore concentrates has received much attention in recent years as an alternative to energy-intensive metal extraction by smelting. A number of promising results have been obtained (e.g., Sakagkuchi, et al., 1976; Torma, 1977; McElroy and Bruynesteyn, 1978; Torma et al., 1979).

The uniformity of the ore in terms of composition and the particle size can affect the rate of leaching significantly. Feed stock from a metal sulfide ore body which is partially oxidized will exhibit a different bioleach rate than feed stock of unoxidized metal sulfide. Solubilizing of the oxidized ore may require only acid leaching whereas solubilization of the metal sulfide will require oxidation.

As regards particle size, boulder-type, ore bearing rock will be leached much more slowly than smaller size ore particles. In general, for low-grade, unbeneficiated ore, there will be a range of particle sizes at which the leach rate is optimal (Torma, 1977; Torma and Guay, 1976; Bruynesteyn and Duncan, 1974). The preferred particle size for any ore will be governed by the distribution of the metal values in the ore. In porphyry-type ore, too much of the metal values will remain locked in the ore if the ore particles are too large . If the particles are too small, the surface area exposed by gangue will be too great in proportion to that of the metal sulfide mineral. Regrinding of ore after leaching has slowed may increase the subsequent rate of leaching (Torma, 1977).

When dealing with ore concentrates, particle size can also affect the rate of leaching, but in this case it has to do with the fact that smaller mineral particles expose a larger surface area for biochemical or chemical attack and thus allow a faster leach rate than larger particles. Even for ore concentrates, there is an optimal particle size for most efficient leaching (Torma and Rozgonyi, 1980; Torma and Guay, 1976).

3.1.5 Special Features of Biohydrometallurgy

3.1.5.1 Metal Isolation From Leach Liquors

Depending on the metal, different methods exist for the recovery of metals from pregnant solution. In the case of Cu^{2+}, precipitation with scrap iron is a favored process,

$$Cu^{2+} + Fe^{o} = Cu^{o} + Fe^{2+}.$$

The resultant copper needs further refining, usually by conventional smelting (Rosenquist, 1974). As an alternative, high-grade copper can be recovered directly from pregnant solution by electrolysis using carbon anodes and copper cathodes (Rosenquist, 1974).

Other metals can be recovered from leach liquor by solvent extraction or electrolytically (Rosenquist, 1974). Electrolysis, although yielding a fairly pure metal product, is energy-intensive and expensive.

3.1.5.2 Recycling of Leach Liquors

Solution mining (heap-, dump-, and in-situ leaching) as applied to metal sulfide deposits is frequently carried out in arid regions where water availability is limited and water has to be conserved. Significant water conservation can be achieved by converting barren solution into leach solution and recycling it on ore heaps or dumps or into mine works for further metal extraction. The conversion of barren solution from a copper sulfide ore leaching process into leach solution in oxidation ponds or special reactors (Zimmerley et al., 1958) not only helps in water conservation, but also is an economic way of generating leach solution. Barren solution from a launder will contain a high concentration of ferrous iron in acid solution resulting from the precipitation of copper from pregnant solution with scrap iron (see sections 3.1.1 and 3.1.5.1). The ferrous iron concentration in such barren solution is usually too high to be directly introduced into a leach dump or ore body leached in-place because when this iron is oxidized, it will give rise to an excess of jarosite precipitate which will passivate ore minerals and plug up flow channels (see 3.1.4.2). By allowing for removal of excess iron by bacterial oxidation and precipitation as jarosite in oxidation ponds or special reactors, the acid level of the solution is raised,

$$6Fe^{2+} + 3/2\ O_2 + 2K^+ + 4SO_4{}^{2-} = 2KFe_3(SO_4)^{-2}(OH)_6 + 6H^+.$$

Some FeIII remains dissolved, the amount depending on the final pH achieved. The extent of oxidation needs to be controlled so that the composition of the leach solution can be optimized with respect to dissolved FeII, FeIII and acidity. Depending on the requirement of the leaching process, the leach solution may need further acidification with an external source of sulfuric acid. As long as the leach solution is not excessively acidified, iron-oxidizing bacteria from the oxidation pond or special reactor will be carried into the leach heap, dump, or ore body and thus reinoculate it. If the number of active bacteria populating the leach heap, dump or ore body is suboptimal, this reinoculation may help to bring it to optimal levels and speed the leaching process.

Oxidation of FeII in barren solution from solution-mining of uranium is another example of recycling, where conversion of FeII to FeIII is important. It is the FeIII in acid solution which is responsible for solubilizing the uranium in uraninite by oxidation, although T. ferrooxidans can also oxidize U(IV) directly (Dispirito and Tuovinen, 1981,1982a). Since FeII is generated in the chemical oxidation of the uraninite, it must be reoxidized to FeIII for further lixiviation of uraninite. In principle, any leach process in which FeIII plays an important role as oxidant in lixiviation can be recycled through oxidation by acidophilic, iron-oxidizing bacteria. Such processes include mostly the leaching of metal sulfides and uraninite ores. A Kennecott patent (Zimmerley et al., 1958) also proposes it for high-grade chromite and titanium-bearing material. It has been shown not to work effectively on chromite ore (Wong et al., 1982).

3.2 RECOVERY OF METALS FROM SEWAGE SLUDGE, WASTE SOLUTIONS AND DILUTE PROCESS STREAMS - METAL CONCENTRATION PROCESSES

3.2.1 Proven Microbial Recovery Processes

Some microbes have been shown to accumulate metal ions on or in their cells, in some cases concentrating them by several orders of magnitude relative to their concentration in solution. In other cases, the microbes have been shown to precipitate metal compounds from solution extracellularly (Brierley and Brierley, 1983; Lundgren and Malouf, 1983) Only a few of these concentration mechanisms are currently being commercially exploited on a major scale although some others have been proposed for this purpose (Brierley and Brierley, 1983). Examples of microbial processes that have been reported to involve significant cellular metal accumulation are summarized in Tables 7 and 8. Other examples include a use of algae in removing metals from an industrial waste stream, as proposed by Spizak (1979). Cork and Cusanovich (1979) have proposed the conversion of waste stream sulfate (for instance acid mine drainage) to native sulfur by Desulfovibrio desulfuricans and Chlorobium thiosulfatophilum. According to Cameron and Koch (1980), anaerobic microbial digestion of trace-metal-containing leachates from landfills has been shown to lead to the precipitation of >85% of Al, Ba, Cd, Hg, Ni, and Zn, 80% of the Fe, 40-70% of the Cr, Cu, Pb, and Mn, 30% of the Ca, and <10% of the Mg, K and Na as insoluble compounds (sulfides) or organic complexes. The metals in the leachate did not affect the anaerobic digestion adversely (Cameron and Koch, 1980). The metals precipitated from the leachate could be extracted from the precipitate by the method of Schonborn and Hartmann (1978). This method involves recovery of the metals, present as sulfides (and probably also as organic complexes), in sewage sludge by enriching the sludge with 1% native sulfur and inoculating with T. thiooxidans, to generate sulfuric acid from the sulfur for lowering the pH, and with T. ferrooxidans, to oxidize the metal sulfides. The metals they solubilized in this way included Fe, Co, Zn, Ni and Cd.

3.2.2 Microbiology of Metal Recovery (Concentration) Systems

3.2.2.1 Types of Microorganisms

As an examination of Tables 7, 8 and 9 will show, types of microorganisms that can be employed in metal recovery systems include bacteria, algae, and fungi. The choice of organism will depend on the source of the metal(s), the metal(s) themselves, the physical state of the metal(s)(whether a precipitate or in solution as free ions or complexes), the nutrient potential of the metal source (Which nutrients are present and how much? Which are lacking? Are any growth inhibitors present and at what concentration?).

Table 7. Microbial metal concentrators

Microbial type	Name of organism	Site of metal accumulation	Types of metal accumulated (and extent)	References
Algae	*Navicula pelliculosa*, *Chlamydomonas*, *C. pyrenoidosa*, *Mongiotia* sp., *S. obliquus*, *Ulothrix fimbrinata*, *Zygnema* sp., *Nostoc* 586, *N. muscorum*, *Oscillatoria*, *S. calcicola*	?	Cd, Pb, Hg (concn. factor of 1,310 to 46,700 at pH 7 depending on alga	Hasselt et al., 1981
	Spirogyra, *Rhizoclonium*, *Cladophora*	Intact cells	Pb (85% removal from a 10 ppm solution	Gale & Wixson, 1979
	Ditylum brightwellii	?	Zn Pb Cu Cd	Canterford et al., 1978
	Porphyra umbilicalis	?	Cd (conc'd 255x)	McLean & Williamson; 1977
	Chlorella vulgaris	Cell surface	U	Horikoshi et al., 1979; Nakajima et al., 1979
	Hormidium fluitans	Cell wall	Ag, Al, Cu, Fe, Mo, Ti, V	Madgwick & Ralph, 1977
Bacteria	*Pseudomonas aeruginosa*	Cytoplasm, cell envelope	Cd^{2+}	Horitsu et al., 1979
	Pseudomonas ambigua G-1	?	Cr(VI)	Horitsu et al., 1978
	Gallionella ferruginia, *Leptothrix* spp. and other sheathed bacteria, *Naumanniella*, *Siderocapsa*	Stalk, sheath, capsule, walls depending on organism	Mn, Fe, depending on organism	Ehrlich, 1981; Lundgren & Malouf, 1983; Silverman & Ehrlich, 1964

Table 7 (continued)

Bacteria	E. coli K-12	Membrane-polar head groups or peptidoglycan layer in wall	Hf, Os, Pb, Zn, Zr, FeIII, Mn, Mo, Mg, Co, CeIV, Na, K, Rb, Ca, Cr Cu, Sc, La, Pr, Sm, U, FeII, Ru, Ni, Hg, Pb, Pt, Pd, Au, In (for extent see Table 10)	Beveridge & Koval, 1981
	Bacillus subtilis	Peptidoglycan layer in wall	Mg, FeIII, Cu, Na, K, Mn, Zn, Cu, AuIII, Ni, Hg, Sr, Pg, Ag (for extent see Table 11)	Beveridge & Murray, 1976
	Bacillus licheniformis	Teichoic and teichuronic acids in wall	Cu, Ni, FeIII, Mn, Ca, Mg, K, Na, Au (for extent see Table 12)	Beveridge et al., 1981
	Pseudomonas aeruginosa	Intracellular	U (10 to 15% of cell dry wt.)	Strandberg et al., 1981
	Zoogloea ramigera	Zoogloeal matrix	Co, Cu, FeIII, Ni, Zn (removed 2.3 to 19 mg per 100 mg of cell, dry wt.)	Friedman & Dugan, 1968
	Pseudomonas fluorescens Citrobacter	Cell surface	Pb (accumulated 1/3 of cell dry wt. as lead phosphate)	Aikin & Dean, 1978
Fungi	Saccharomyces cerevisiae	Cell surface	U (10 to 15% cell dry wt.)	Strandberg et al., 1981
	S. cerevisiae, S. uvarum, S. lipolytica, C. utilis, Rhodotorula mucilaginosa,	Intact cells	Co, Cd, Zn, Ni, Cu, Mn	Norris & Kelly, 1979

Table 7 (continued)

Fungi	*Aspergillus niger*	Chitosan-glucan (wall polymers)	Cu, Hg	Muzzarelli et al., 1980
	Penicillium digitatum	Chitin (cell wall)	UO_2^{2+} (10,000 ppm by dry wt.)	Galun et al., 1983

Table 8. Biological processes for metal removal from sewage, leachate, wastewater, etc.

Type of process	References
Removal of iron from solution by *Chlorella vulgaris* and *Scenedesmus quadricauda*	Bagnyuk et al., 1976
Removal of Mo and U from uranium mine waste by algae (*Chara*, *Spirogyra*)	Brierley et al., 1980
Removal of Cd and Ni by *Chlorella fusca* and *Scenedesmus obliquus*	Brueckner and Hoefner, 1979
Bioaccumulation of silver by bacteria	Chaley and Bull, 1979
Cd uptake by bacteria	Doyle et al., 1974
Metal accumulation by fuel-utilizing bacteria	Engel and Owen, 1970
Removal of As from wastewaters by iron bacteria in lagoons	Enker et al., 1980
Cd, Cu, Cr removal by algae in wastewater lagoons (lab scale study)	Filip et al., 1979
Ag removal from waste photographic processing solutions containing reductive sulfur compounds with the help of photosynthetic bacteria	Fuji Photo Film Co., Ltd., 1979
Cyanide removal from waste streams by bacteria	Ilyaletdinov et al., 1977
Removal of heavy metals from effluents by heterotrophs growing at the expense of plant residues (e.g., cattails)	Ilyaletdinov et al., 1976
Accumulation of Cr by periphytic bacteria on carapace and gills of crabs near a tannery effluent outlet	Johnson et al., 1981
Cd accumulation by *Citrobacter* sp.	Macashic and Dean, 1982
Biochemical removal of Cr(VI) from wastewater with activated sludge	Pleshkov et al., 1981
Bioaccumulation of Ag as Ag_2S on bacterial cell surfaces	Pooley, 1982

Table 8 (continued)

Biochemical removal of Cr(VI) from wastewater with activated sludge	Romanenko et al., 1976
Acceleration of biological removal of Cr(VI) by use of perlite-lignosulfonate as 1.0 to 2.5 nm porous granules with bulk density of 0.3-0.9 kg/dm^3	Serpokrylov et al., 1982
Removal of uranium from aqueous solution by a mixed culture of denitrifying bacteria	Shumate et al., 1980
Metal removal by adsorption and precipitation in an activated sludge process	Sterritt et al., 1981
Removal of heavy metals by *Chlorella* and *Scenedesmus* accumulation from effluent of a nonferrous metals plant in Bulgaria	Todorova-Trifonova et al., 1979
Co removal by genetically engineered bacteria	Wong and Williams, 1982
Metal removal by sulfate-reducing bacteria from wastewater	Zainullin et al., 1981

Table 9. Bacterial metal precipitation without accumulation in or on cells

Name of organisms	Metal(s) precipitated	Mechanism of precipitation	References
Desulfovibrio, Desulfotomaculum	U, Se, Mo, Cu, Fe, etc.	Reduction and precipitation by biogenic H_2S	Miller, 1950; Baas Becking and Moore, 1961; Temple and LeRoux, 1964; Brierley and Brierley, 1980; Zainullin et al., 1981
Vibrio, Oceanospirillum, Arthrobacter, other bacteria	Mn	Enzymatic oxidation	see Ehrlich, 1981
Thiobacillus ferrooxidans, Sulfolobus, and other bacteria	Fe	Enzymatic oxidation	see this report

3.2.2.2 Types of Microbial Concentration Processes in Terms of Physiological Mechanisms

1. Adsorption to specific cell components.

As a perusal of Tables 7 and 8 will show, many reported metal concentrating processes by bacteria, algae, and fungi involve passive binding by one or more cell components with a special affinity for metals. Cell-surface components include cell-wall polymers like peptidoglycans, teichoic and teichuronic acids, and slime- layer polysaccharides in prokaryotes, chitin and probably some other glycans of fungal cell walls, and unidentified polymers in algal cell walls. Intracellular components include various proteins, especially metallothioneins. The affinity for metal cations of these polymers is owing to negatively charged groups (chiefly carboxyl groups and phenolic hydroxyl groups) and also sulfhydryl groups (-SH). The affinity for metal anions is mainly due to protonated amino groups.

Some eukaryotic microbial cells as well as some prokaryotic ones produce metallothioneins. These are low-molecular weight, intracellular proteins which are very rich in sulfhydryl groups and, therefore, have a strong affinity for heavy metal cations (Vasak and Kaegi, 1983). In prokaryotes, metallothioneins have so far been found only in cyanobacteria (Olafson et al., 1980).

All these metal binding processes are passive in the sense that a cell need not do work to bring metal-binding about. Metal-binding occurs because the ligand has a higher affinity for the metal ion than the solvent (normally water) from which it is removed.

2. Metal binding to a cell component after oxidation

Some microorganisms bind Fe and/or Mn to their cell surface after oxidizing either FeII of MnII to FeIII or MnIV, respectively, or after freeing FeIII from a ligand such as humate (see Ehrlich, 1981). Gallionella ferruginea can grow autotrophically on FeII in a partially reduced environment, depositing the ferric iron as as oxide in its lateral stalk (Hanert, 1981b). Leptothrix ochracea and other species of Leptothrix have been reported to oxidize FeII or MnII and deposit the corresponding oxides in their sheaths (see Ehrlich, 1981; Lundgren and Dean, 1979). It is not certain at this time how Leptothrix spp. oxidize FeII and whether they can obtain energy from the oxidation of either FeII and MnII. Encrustation of the sheath by either iron or manganese oxide may be so heavy as to completely obscure it.

Members of the family Siderocapsaceae may be able to oxidize FeII or MnII. Even if incapable of oxidizing these substances, they accumulate the FeIII or MnIV oxides in their capsules (Hanert, 1981a).

3. Iron fixation as magnetite in bacterial magnetosomes

Some bacteria deposit iron in special vesicles as magnetite. The size of the magnetite crystals is restricted by the cell. The crystals impart magnetotactic properties to the cells, i.e., the cells can employ them as a compass which directs them to their preferred habitat in nature, namely an environment with low oxygen- tension. The mode of formation of the magnetite crystals is still unknown (see Blakemore, 1982 for detailed information). The magnetite crystals have very useful physical properties. They may be produced en masse by culturing the bacteria which produce them in large quantities. A patent has been issued to Blakemore, the discoverer of these bacteria, for their commercial use (Blakemore, 1983).

4. Extracellular metal precipitation after bacterial oxidation or reduction

As already described (see 3.1.5.2), acidophilic, iron oxidizing bacteria can precipitate iron by oxidizing FeII to FeIII which then precipitates as basic ferric sulfate (jarosite) above pH 2.0 (Lazaroff et al., 1982)

A number of bacteria from soil, freshwater and marine environments have been shown to oxidize MnII to Mn(IV) oxide (see Ehrlich, 1980, 1981). The oxidized manganese does not precipitate on the cells in these cases. Many of the bacteria can oxidize free Mn^{2+}, but some marine bacteria can only oxidize Mn^{2+} which has been bound to Mn(IV) oxide, ferromanganese, unidentified marine clay fractions, or montmorillonite or kaolinite types of clays (Ehrlich, 1981, 1982, 1983). The oxidized manganese remains bound to the sorbent.

A strain of Pseudomonas fluorescens and some other Pseudomonas strains can reduce CrVI to CrIII which precipitates as $Cr(OH)_3$ at neutral pH. The organisms use glucose as electron donor in an enzymatic process (Romanenko and Koren'kov, 1977; Bopp, 1980; Bopp and Ehrlich, 1981).

5. Metal precipitation with biogenic H_2S

Heavy metals can also be precipitated by H_2S generated by sulfate-reducing bacteria (see Ehrlich, 1981). Besides the classical Desulfovibrio and Desulfotomaculum strains (Postgate, 1979), a variety of sulfate-reducing bacteria have been discovered in the last few years. They differ in part with respect to the energy sources they utilize (Pfennig et al., 1981). The organisms include autotrophic, hydrogen-utilizing sulfate-reducers which use acetate as reductant.

6. Metal precipitation by oxidation or reduction by metabolic

products

MnII may be precipitated as Mn(IV) oxide at a pH of around 5.0 by an as yet unidentified secondary metabolite produced by a Streptomyces sp. isolated from soil (Bromfield, 1978, 1979). MnO_2 or other Mn(IV) oxide may be reduced by metabolically produced formic and/or oxalic acid (Ehrlich, 1981a). Formic acid is produced by some bacteria like Escherichia coli. Oxalic acid is produced by some fungi like Aspergillus niger. The reduced manganese may be reprecipitated as carbonate by biogenic CO_2.

3.2.2.3 Regulation of Metabolism

Not much specific work on the regulation of metabolism relevant to metal accumulation by microbial cells has been done. Passive metal accumulation by microbial cells is not likely to involve metabolic control. However, the formation of bacterial slime, which may have an affinity for metal ions, may depend on the availability of specific nutrients such as certain sugars. The manganese oxidase of Leptothrix ochracea (also known as Sphaerotilus discophorus), whose activity is needed for accumulation of Mn(IV) oxide in its sheath, is inducible. Similarly, the manganese oxidase of several as yet unidentified marine bacteria from hydrothermal vent communities in mid-ocean rift zones on the ocean floor, which deposit Mn(IV) oxides in their environment, is inducible (Ehrlich, 1983a).

3.2.2.4 Role of Inheritance and Environmental Factors

According to Silver (1983), bacteria sensitive to toxic heavy metals are incapable of pumping such toxic metals out of the cell, before they are bound, if at all. Metal accumulation kills them. Bacteria resistant to such heavy metals mostly develop specific, genetically determined pumping mechanisms in their cell membranes which expel these metals as fast as they enter. Entry by many of the toxic metals appears to be by way of gentically determined, membrane-transport systems which are actually designed for transporting non-toxic, required inorganic substances. Energy is required in many instances to enable transport of toxic or non-toxic substances. Uptake of essential metals, such as Mg^{2+}, Fe^{2+}, Cu^{2+}, etc. by cells is limited by need. This limitation is determined by the uptake kinetics of the different metal ions. It stands to reason, therefore, that wild-type, metal-sensitive bacteria or their metal- resistant mutants are not useful for accumulating metals intracellularly, except in cases where low-molecular weight metallothioneins or other similar substances are formed to tie up the metals after uptake.

Significant accumulation of toxic metals by microorganisms is, therefore best accomplished by passive mechanisms. Genetics enters into the picture only insofar as it determines the cellular structures which bind the metals.

3.2.2.5 Mutualistic Associations

Kramarenko (1962) reported the detection of various thiobacilli in underground waters of Kazakhstan-rare metal-molybdenum deposits, including T. thiooxidans, T. ferroxidans, T. denitrificans, and T. thioparus. He also detected sulfate-reducers and other heterotrophic bacteria. The type of sulfide mineral with which the groundwater was in contact affected the microbial types present in it. Sulfate-reducers kept molybdenum precipitated.

Groudev et al., (1978) have described a complex community of microorganisms, including acidophilic and neutrophilic thiobacilli, iron bacteria, heterotrophic bacteria, yeasts, fungi, protozoa, and algae, in part similar to earlier findings by Ehrlich (1963). Some of their bacteria were thermophiles capable of leaching at elevated temperatures (60° C). Some can have a suppressive effect on the thiobacilli by competing for oxygen by producing inhibitory substances, or in the case of amoebae, consuming thiobacilli as food. The make-up of the microbial community can vary in different parts of a cyclic dump or an ore body leached in-place.

Tsuchiya et al. (1974) reported that growth of Thiobacillus ferroxidans ATCC 19361 is enhanced by the presence of Beijerinckia lacticogenes ATCC 19361 when no external carbon or nitrogen sources are supplied in the growth medium (see also section 3.1.2.4). This enhancement also manifested itself in acceleration and extent of leaching of Cu and Ni from an ore concentrate.

Pooley (1982) has reported that a mixed culture of T. ferrooxidans and T. thiooxidans accumulated Ag_2S (acanthite) on the cell surface amounting to up to 25% of the cell dry weight when leaching a silver-containing sulfide mineral. This silver could be recovered by cyanidiation. It is not clear whether the simultaneous presence of T. ferrooxidans and T. thiooxidans was required to deposit the silver as sulfide. No silver sulfide was deposited when just dissolved silver and ferrous sulfate were available.

Although not well studied as yet, evidence exists for enhanced metal accumulation by mixed cultures as compared to pure cultures (Brierley and Brierley, 1983). For instance, biological removal of metals from mining effluents as at Flin Flon in Canada was optimized by the cooperative action of algal blooms promoted by introduction of sewage effluents and sulfate-reducing bacteria, the latter living at the expense of the decomposing algae (Jackson, 1978). The algal cells in the blooms initially scavenged the metals such as Zn, Cd, Cu, Hg, and Fe. On die-off, the settling algal cells carried the metals to the bottom. The algal cells underwent mineralization (conversion into inorganic matter) with sulfate-reducing bacteria completing the metabolic chain of events. The hydrogen sulfide which the sulfate-reducers generated helped to fix the metals as sulfides in the sediments. Similar happenings are suggested by the work of Brierley and Brierley (1980) in connection with mining effluents containing U, Mo, and Se.

3.2.3 Mechanistic Studies

Metal uptake by cells may involve cell surface structures and/or the cell interior. In general when intact cells are considered, the cell surface can accommodate larger quantities of a metal than the cell interior, although exceptions are known (e.g., Strandberg et al., 1981). This is explained by the nature of the metal-deposition mechanism at the cell surface and the nature of the mechanism of metal penetration into a cell, as will be explained in sections 3.2.3.1 and 3.2.3.2. When considering intact cells for metal concentration, therefore, surface binding is usually a more efficient process. On the other hand, many eukaryotic cells and at least some prokaryotic cells produce low-molecular weight proteins rich in cysteine called metallothioneins, which have a high affinity for heavy metals. If cells containing metallothioneins are broken and their metallothioneins released and artificially bound to a polymer support, an even more effective metal concentrator may be available than cell surfaces or isolated cell-surface polymers with metal affinities.

3.2.3.1 Surface Adsorption

As revealed in section 3.2.2.1, a number of bacteria, yeast and other fungi, and algae exhibit a special ability to concentrate metals from solution in their cell surface. In the case of bacteria, Beveridge and Koval (1981) showed that Escherichia coli K-12, a gram-negative bacterium, bound metals mainly to polar head- groups of cell membranes or to peptidoglycan of the cell envelope. They found an affinity for metals as shown in Table 10. Beveridge and Murray (1976) showed that Bacillus subtilis (gram-positive) also bound metals to its cell wall (Table 11). The peptidoglycan component of the B. subtilis wall was the main site of binding (Beveridge and Murray, 1980). By contrast, Beveridge et al. (1982) found that surface binding of metals by Bacillus licheniformis (gram-positive) involved the teichoic and teichuronic portions of its cell wall (Table 12).

Interesting processes of metal removal have been reported for denitrifying bacterial systems. Tengerdy et al. (1980) investigated the ability of Pseudomonas aeruginosa immobilized on polyvinyl chloride or polypropyline plastic surfaces to remove heavy metals while denitrifying. They found that such cells could reduce a lead nitrate concentration in a feed solution from 1 mg/L to 0.05 - 0.1 mg/L and a zinc sulfate concentration from 10 mg/L to 5 mg/L. In a batch system, cells immobilized on polypropylene, could remove 96% of a 1.7 nCi $PuCl_4$ activity.

Shumate et al. (1980) found that a mixed culture of denitrifying bacteria could reduce a uranium concentration from $100 g/m^3$ to about $3 g/m^3$ in 15 min at 25 to 50 degrees Celsius. In a continuous counter current system, a uranium concentration of $25 g/m^3$ was reduced to $0.5 g/m^3$ in 8 min. Although mentioned here as an example of extracellular uranium accumulation, it could be an intracellular process (see the case for Pseudomonas aeruginosa in 3.2.3.2).

In the case of yeast, Strandberg et al. (1981) reported that Saccharomyces cerevisiae (about 0.4 g by dry weight per ml) bound uranium $UO_2(NO_3)_2$, which it had taken up from dilute solution (ca. $100 g/m^3$ at 40 degrees Celsius), on its cell surface to the

Table 10. Metal affinity of purified *Escherichia coli* K-12

Metals	Binding range* (umol of metal per mg cell envelope)
Hf, Os	0.9
Pb, Zn, Zr, FeIII, Mn, Mo, Mg, Co, CeIV	0.1 -0.4
Na, K, Rb, Ca, Sr, Cu, Sc, La, Pr, Sm, U, FeII, Ru, Ni, Hg, Pb, Pd, Au, In	0.1
Li, V	0

* 5 mg (dry wt) of cell envelopes were incubated in 2 ml of 5 mM metal solution under standard conditions.

Reference: Beveridge and Koval, 1981

Table 11. Metal affinity of Bacillus subtilis wall fragments

Metals	Binding range* umol of metal per mg of wall fragments
Na, K, Mg, FeIII, Cu	1.0
Ca, Mn, Ni, AuIII, Zn, Sr, Pb	0.1-0.8
Sr, Pb, Ag, Hg	0.1
Li, Ba, Co, Al	0

* 5 mg (dry wt) of cell wall fragments were incubated in 2 ml of 5 mM metal solution for 10 min at 22 C

Reference: Beveridge and Murray, 1976

Table 12. Metal affinity of native wall preparations of _Bacillus licheniformis_

Metals	Binding range* umol of metal per mg of wall preparation
Na, K, Mg, Cu, Mn, FeIII, Ni, Ca	0.4-0.9
Au	0.1

* Incubated 1 mg of wall preparation in 2 ml of 5 mM metal solution

Reference: Beveridge et al., 1982

extent of 10 to 15% of the cell dry weight. The authors suspected that the uranium became bound to the phosphomannans of the cells. The uptake was not dependent on metabolism, but the rate and extent of accumulation were influenced by pH, temperature, and certain anions and cations. Bound uranium could be released to varying degrees with 0.1 M nitric acid, 0.1 M ethylenediaminetetraacetic acid (EDTA), or 0.1 M ammonium carbonate. The cells were reusable for uranium uptake after elution.

Filamentous fungi have also been found useful for recovering radionuclides like uranium, thorium, and radium from solution (Tsezos and Volesky, 1981, 1982a,b; Tsezos and Keller, 1983). Rhizopus arrhizus has been shown to have uranium and thorium uptake capacity in excess of 180 mg/g (dry weight) of mycelium at pH 4, as established from biosorption isotherms. This was significantly better than the adsorption capacity of anionic exchange resin (IRA-400 of Rohm and Haas Co.) or activated carbon Filtrasorb 400 (Calgon Co.)(Tsezos and Volesky, 1981). Uranium and thorium binding by the mycelium was very pH dependent (pH 4-5 was optimal). Uranium taken up by R. arrhizus mycelium was sequestered by the cell envelope by at least three processes: (1) rapid coordinating by the nitrogen groups of the cell-wall chitin, (2) rapid adsorption to the cell wall chitin, and (3) slow precipitation as uranyl hydroxide within the chitin microcrystalline wall structure (Tsezos and Volesky, 1982a). Fe^{2+} and Zn^{2} interfered with uranium uptake. Thorium taken up by R. arrhizus was also sequestered in its cell envelope, but in this case involving only (1) coordination with the nitrogen groups of chitin and (2) adsorption to the external section of the cell wall. Fe^{2+} and Zn^{2+} did not interfere significantly in this case (Tsezos and Volesky, 1982b).

Radium was found to be concentrated by the fungus Penicillium chrysogenum. Radium uptake by this organism was optimal in the range of pH 7 to 10. The uptake capacity of the fungus was $5x10^{4}$ nCi/g dry weight as compared to activated carbon

(Filtrasorb 400)(3,600 nCi/g) at an equilibrium concentration of 1,000 pCi/L at pH 7 (Tsezos and Keller, 1983).

Chitin in the walls of fungi and the exoskeleton of arthropods does not only have an affinity for U, Th, and Ra, but also for other heavy metals (Yoshinara and Subramanian, 1976; Muzzarelli, 1977). Purified chitin from lobster shells exhibited some selectivity toward heavy metal ions (Zn>Ni>Co>Cu>Pb) according to Yoshinara and Subramanian (1976). Muzarelli (1977) has observed that transition metals of the first series (Ti, V, Cr, Fe, Co, Ni, and Cu) are bound by purified chitin and chitosan (deacetylated chitin) from fungi, forming variously colored complexes. Fungal chitosan exhibits differential affinity for metal cations. Sulfate anions enhance metal uptake while chloride anions suppress it. Conditioning of chitosan can enhance its adsorption capacity. Elution of sorbed metals is said to be easy. However, FeIII can only be eluted after reduction to FeII (Muzzarelli, 1977). In general, the mechanism of binding of metal cations is thought to be through formation of coordination complexes with $-NH_2$ groups of conditioned chitosan. Organisms of metals react by anion exchange on protonated amino groups of conditioned chitosan (Muzzarelli, 1977). Unconditioned chitosan probably binds metal ions by ion exchange sorption and chelation (Muzzarelli, 1977). Chitosan-glucan complex (200 mg of 100 to 200 mesh powder) from the fungus Aspergillus niger bound up to 98% of Cr^{3+}, 82% of Mn^{2+}, 96% of Co^{2+}, 98% of Ni^{2+}, 100% of Cu^{2+}, 90% of Zn^{2+}, 90% of Cd^{2+}, and 100% of Pb^{2+} from 50 ml of 0.5 mM metal sulfate solutions (except for lead nitrate) at pH 5.6 (final pH 7.5) after 1 h of shaking at room temperature (Muzzarelli et al., 1980).

Insofar as metal uptake by algae is concerned, Gale and Wixson (1979) tested washed field specimens of filamentous algae (Spirogyra, Rhizoclonium, Cladophora) in chromatographic columns (1 to 2 cm in diameter) with about 2 L of lead acetate solution, containing 10 ppm of lead at neutral pH, at a flow rate of 3 to 8 ml/min. They found 85% of the lead to have been taken up. The bound lead could be removed by washing with 0.01 to 0.1 M EDTA in 0.1 M sodium acetate at pH 7.5. Examination of ultrathin sections of the algae revealed the lead to have been accumulated on and within the cell walls and capsular material and occasionally in membrane folds, vesicles, mitochondria, and chloroplasts with the cytoplasm.

Ferguson and Bubela (1974) have studied Cu, Pb, and Zn uptake by particulate organic matter from the green algae *Ulothrix, Chlamydomonas, and Chlorella*. They measured enrichment factors for copper ranging from 3,300 to 9,700, for zinc ranging from 700 to 970, and for lead of 25,400 (*Ulothrix* only). They found that even low concentrations of Na and Mg ions in solution lowered the amount of Zn taken up to near zero, but Pb and Cu adsorption was largely unaffected.

3.2.3.2 Intracellular Uptake of Metals

In general, metals which are required nutritionally (e.g., Co, Cu, Fe, Mg, Mn, Mo, Ni, Se) by microbial cells are taken up by special mechanisms which make possible their passage through the cell membrane (Silver, 1978; Silver and Perry, 1982). Since at high concentration, these metals are toxic to the cell, their uptake must be carefully regulated by these mechanisms. Such regulation may be accomplished by

1. requiring energy to be expended to pull a particular metal ion into the cell (active transport) (Kobayashi et al., 1980);

2. by requiring an exchange in which an entering metal ion requires the simultaneous exit of another cation of a different species (antiport) (Beck and Rosen, 1979);

3. by requiring the simultaneous entry of another specific but different molecule (organic or inorganic) into the cell (symport) (Simoni and Postma, 1975);

4. by requiring a special passive (i.e., non-energy requiring mechanism) to draw a metal ion into the cell (facilitated diffusion).

Hardly ever do metal ions enter a healthy, living cell by simple diffusion. Swamping a cell interior with a metal, which though needed but toxic in excess, is thereby prevented. Somehow, cells do not take up more of a needed metal than is required for normal functioning. In the case of at least some metals, it has been shown that special export mechanisms which specifically pump metals from the inside of a microbial cell to the outside exist. They help to explain how a build-up of metal in a cell can be prevented (Silver, 1983; Perry and Silver, 1982; Tynecka et al., 1981).

Some transport systems for nutritionally required metals (e.g., Mn^{2+}) may also transport unneeded and toxic metals (Cd^{2+}), which may thus compete for entry with the required metals. Cells that have some level of resistance to these toxic metals when taken into the cell, export them by a specific exporting mechanism (Perry and Silver, 1982, Silver, 1983).

In cases where an internal metal concentration is restricted by a specific efflux mechanism, it should be theoretically possible to cause swamping of the cell interior if the efflux system is eliminated by genetic manipulation. In practice, however, the metal, especially if toxic, may quickly reach an internal concentration which poisons the system that pulls the metal into the cell.

An exceptional case of relatively large metal uptake into the cell interior of Pseudomonas aeruginosa has been described by

Strandberg et al. (1981). Ps. aeruginosa apparently can accumulate 100 g of uranium in 10 sec. For this experiment, 0.4 mg (dry weight) of cells per ml of uranyl nitrate (100 g of uranium per m^3) were tested at 40 degrees Celsius. The cells did not have to be actively metabolizing to take up the uranium. The accumulated uranium could not be extracted from the intact cells.

3.2.3.3 Specificity in Metal Accumulation By Microorganisms

Most intact cells, whether prokaryotic or eukarkyotic, do not show great selectivity in the binding of heavy metals. Major exceptions are certain iron- and manganese-bacteria. Gallionella ferruginea, for instance, deposits large amounts of ferric oxide in its lateral stalk, the oxide being a product of its energy metabolism of FeII (Hanert, 1981b). The organism does not deposit manganese oxides. Some sheathed bacteria (Leptothrix spp.) deposit major quantities of either iron or manganese oxides in their sheath but no significant amounts of other metals (Mulder and Deinema, 1981). The oxides may or may not be a product of their metabolism. Other iron bacteria, like the Siderocapsaceae, deposit extensive amounts of ferric or manganese oxide in their capsules (Hanert, 1981a). Whether this deposition involves iron and manganese metabolism is not fully understood at this time. The lack of great specificity in metal binding by the surface structure of other intact microorganisms may be attributable to the existence of several different binding compounds of varied specificity, a lack of specificity of the main binding compound, the conditions for binding, or a combination of these factors.

Isolated biopolymers from cell surfaces, like chitin especially after conversion to chitosan by deacetylation in concentrated NaOH solution, can be made specific by further pretreatment and by carefully controlling the conditions of metal sorption (Muzzarelli, 1977). The basis for this is the different affinities for different metal cations of the chitosan preparations, and the fact that chitosan can be transformed into an anion exchange resin which has an affinity for metal oxyanions but not cations (Muzzarelli, 1977).

Intracellular metal-ion uptake by intact cells is specific because in most cases special transport mechanisms are involved. But cellular uptake need not be selective in a mixture of metal ions because several of these uptake mechanisms may function simultaneously. Metal ion uptake into the cells may be made more specfic by genetic manipulation. Significant intracellular accumulation will require the elimination of any efflux of the metal being accumulated.

3.2.3.4 Practial Applications

Bioaccumulation of metals by intact cells is exploited practically at the present time in very dilute metal solutions (waste streams, polluted waters, etc.). It is not presently exploited in the large-scale recovery of metals in leaching processes. A major reason is the large biomass requirement for significant metal concentration.

To be commercially attractive, a bioaccumulator, whether intact cell or an isolated cell component, (1) must have a high selective affinity for metals, (2) be readily and cheaply producible, (3) be reusable after elution of the concentrated metal, (4) be chemically stable, (5) and be cheaply disposable without causing a pollution problem after its usefulness is exhausted. Although peptidoglycan of bacterial cell walls (see Tables 10 and 11) and chitosan (see Muzzarelli, 1977) seem to bind equivalent amounts of Cu^{2+} (in the order of 50 kg of Cu^{2+} per tonne), chitosan is so much easier to prepare on a large scale and its source material, fungal mycelium, so readily available in large quantities as a byproduct of the fermentation industry, that by the above criteria it should be preferable commercially as a bioaccumulator. Muzzarelli (1977) estimated the cost of production of chitosan in 1977 to be between $1.98 and $4.84 per kg, which is very expensive unless chitosan can be reused a great many times.

3.3 EXTRACTION OF METALS BY HETEROTROPHS

Although autotrophs have been most extensively considered for the commercial extraction of metals from ores and metal-containing wastes, the possibility of using heterotrophs exists. The reason for favoring autotrophs in metal extraction from ores and metal-containing wastes is that autotrophs do not require organic nutrients, which may represent costly reagents in the leaching process. Furthermore, they do not generally require strict control of asepsis (prevention of microbial contamination). On the other hand, a disadvantage to using autotrophs in metal or metal-containing waste extraction can be their relatively slow rate of growth compared to that of many heterotrophs (doubling times of 4-12 h or more for autotrophs versus 20 min to 2 h or more for heterotrophs).

Heterotrophs can be especially useful in ore extraction, when autotrophs cannot act for biological reasons. For instance, some metal sulfide ores may have too high a carbonate content such that the high acidity required for the action of *T. ferrooxidans* and/or *T. thiooxidans* cannot be maintained or requires so much pretreatment with sulfuric acid that leaching with *T. ferrooxidans* becomes uneconomic. In that instance, it may be possible to leach with the help of fungi such as *Penicillium* spp. or *Aspergillus* spp. growing in glucose- or sucrose-containing medium (Wenberg et al. 1975; Hartmannova and Kuhr, 1974; Kiel, 1977). Bacteria (*Serratia marcescens, Agrobacterium tumefaciens, Bacillus subtilis, B. Sphaericus,* and *B. firmus*) have been reported to solubilize copper and gold as well as some other metals contained in laterites and clays (Pares, 1964a,b,c). Gold in sulfide ores has also been found to be solubilized by bacteria (Bacillus spp., Pseudomonas spp.)(Korobushkina et al., 1983). In all these cases, the mechanism of solubilization appeared to be through production of complexing agents such as citric, oxalic, 2-ketogluconic, lactic, glycollic, and amino acids.

Metal extraction by heterotrophs may involve reduction instead of oxidation of the metal being extracted. For instance, Ehrlich et al. (1973) have shown that a marine bacterium such as Bacillus GJ33 can reduce the Mn(IV) oxide of marine ferromanganese nodules using glucose as reducing agent. By reducing the Mn(IV) oxide, the bacterium brings Mn, Cu, Ni, and Co but not the Fe of a nodule in solution. Trimble and Ehrlich (1968) showed that a mixed culture from a ferromanganese nodule solubilized 2.6 g or about 10% of the manganese from a 17.6g Atlantic ferromanganese nodule fragment in 16 days in 1 liter of seawater enriched with 0.5% glucose and 0.05% peptone. Similarly, ferric oxides can be reduced and solubilized by bacteria with glucose as electron donor (Munch and Ottow, 1980, 1982).

In general, it can be stated that heterotrophic bacteria can extract metals by direct or indirect action. In direct action, they enzymatically attack the metal moiety of the ore. This usually involves biochemical reduction in which the metal moiety acts as terminal electron acceptor in place of oxygen in a respiratory process, the metal being much more soluble in a reduced form. In indirect action, the heterotroph may produce a metabolic product which reacts chemically (non-enzymatically) with the metal moiety of the ore by doing one or more of the following:

1. reducing it to a lower and more soluble valence state;

2. forming a soluble complex which is more stable than the metallic mineral in the ore;

3. solubilizing the metal moiety by acidifying the reaction medium.

To be useful in ore extraction, an enzymatic process must be a dissimilatory process, i.e., one in which the metal moiety is a reactant in an energy generating process. That is because a cell needs lots of energy for growth and thus must use a lot of the metal reactant to meet this requirement. By contrast, in an assimilatory process, while a metal moiety like ferric iron may also be solubilized and reduced , the quantity of iron involved is very small, - only as much as the cell needs for incorporation into a specific cellular component like cytochrome and/or Fe-S proteins.

By the same token, to be useful in ore extraction, a non-enzymatic solubilizing process must involve a metabolic product which is produced in large quantities by the cell, usually in its metabolism of an organic energy source. However, in some cases it may also be a secondary metabolite. Table 13 summarizes heterotrophic activities applicable to mineral extraction.

Table 13. Heterotrophic activities applicable to mineral extraction

Class of process	Type of reaction	Organism	References
Non-enzymatic	Complexation of Cu, Fe, Au	*Serratia marcescens*, *B. subtilis*, *B. firmus*, *B. sphaericus*	Pares, 1964a
	Complexation of Au and Cu; precipitation of Fe	*Serratia marcescens*	Pares, 1964b
	Complexation of Au	*Agrobacterium tumefaciens*	Pares, 1964c
	Solubilization by complexation and acidification of Al, Fe, and Mg	*Penicillium simplicissimus*	Silverman & Munoz, 1970
	Solubilization by complexation and acidification of Li, Al, Fe	*Trichoderma lignorum*, *Penicillium notatum*	Avakyan et al., 1981
	Solubilization by acidification of Li, Al		Karavaiko et al., 1980
	U(VI) removal from solution	*Penicillium digitatum*	Galun et al., 1983
	Complexation of Au by amino acids	*Bacillus* spp, *Pseudomonas* spp.	Korobushkina et al., 1983
	Solubilization of Cu by acidification and complexation	*Penicillium* sp.	Wenberg et al., 1971
	Solubilization of Cu by acidification and complexation	*Pencillium* sp. *Aspergillus* sp.	Hartmannova & Kuhr, 1974
	Removal of Cu, Zn, Fe from solution	*Aspergillus niger*, *A. amori*	Timanov et al., 1982
	Solubilization by complexation (?) of PbS, ZnS, CdS, CuS	Unidentified soil microbes	Cole, 1979
	Complexation of Au by amino acids	*Bacillus mesentericus*	Korobushkina et al., 1976

Table 13 (continued)

Non-enzymatic	Solubilizaton of Cu by complexation and acidification	*Aspergillus niger*	Kiel, 1977, Kiel and Schwartz, 1980
Enzymatic	Reduction of Mn(IV) oxides	*Bacillus* 29 and other bacteria	Trimble and Ehrlich, 1968
	Reduction of Mn(IV) oxides	*Pseudomonas* sp., *Bacillus* sp., *Arthrobacter* sp.	Agate & Deshpande, 1977
	Reduction of Mn(IV) oxides	Unidentified bacterium; mixed culture	Mercz & Madwick, 1982
	Reduction of ferromanganese with concurrent release of Cu, Ni, and Co	*Bacillus* GJ 33	Ehrlich et al., 1973
	Solubilization of Mn in mangnese ores	*Bacillus* sp.	Perkins & Novielli, 1962
	Reduction of FeIII oxides	*Clostridium butyricum*, *Bacillus polymyxa*	Munch and Ottow, 1980, 1982

4.0 NEW RESEARCH AND TECHNICAL DIRECTIONS

4.1 CURRENT ACTIVITIES AND INITIATIVES

4.1.a Bioleaching of Copper Sulfide Ores

4.1.a.1 Dump-, Heap-, and In-Place Leaching of Low-Grade Ores

Bioleaching of copper from low-grade ores or mine wastes in dumps, heaps, or in-place by acidophilic, iron-oxidizing bacteria, the oldest applications of biohydrometallurgy, is a continuing practice. Since bioleaching of low-grade ores is still an empirical science, each new ore type has to be tested for bioleachability before full-scale dump or heap bioleaching is undertaken. This type of leaching requires little manpower and no special equipment except in the stripping of metal values from pregnant solution. Despite slow rates of leaching, it is cost-effective.

4.1.a.2 Bioleaching of Copper-Sulfide Ore- Concentrates

Detailed investigations have been and continue to be made in bioleaching of ore concentrates for the recovery of copper from chalcopyrite and other types of copper mineralizations. This type of leaching requires special equipment. While technologially feasible, large-scale commercial application is dependent on a fast leach rate and/or the recovery of a very valuable by-product to pay for the cost of the process and allow for a reasonable profit. In the eyes of mineral processors, these conditions have not yet been fully met on a laboratory- or pilot-plant scale to be competitive with conventional treatments (smelting, etc.). The question does arise, however, whether the best currently developed processes for bioleaching of concentrates might not be competitive in situations where conventional methods of concentrate processing are not available (remote ore bodies worked by an established company; or in a developing nation with few or no ore processing facilities, etc.).

4.1.b Bioleaching of Non-Cupriferous Metal-Sulfide Ores

Extensive investigations into leaching of non-cupriferous metal sulfide ores have been and continue to be undertaken, mainly on a laboratory scale. Sulfide ores containing one or more of the following metals, Zn, Ni, Mo, Pb, Co, Ga, In, Tl, Sn, As, Sb, and Se, have been successfully leached in the laboratory. However, no large- scale exploitations seem to have been made as yet of these processes, either as applied to low-grade ores in heaps, dumps,or directly to broken ore bodies, or to ore concentrates. The reasons for this lack of exploitation are not entirely clear but may involve too low a leaching rate and/or too costly a metal recovery process from pregnant solutions. Recovery methods would include differential precipitation, solvent extraction, adsorption to an ion exchange resin, electrolysis, etc.

4.1.b.1 Galvanic Effects on Microbial Leaching of Sulfide Ore

Since metal sulfide minerals can act as electric conductors, and since they may exhibit different resting protentials, depending on the kind of mineral, pairs of them in contact with an aqueous solution may form a galvanic cell. The mineral with the lower resting potential would exhibit the greater tendency to oxidize and dissolve (Berry et al., 1978; Mehta and Murr, 1982). Sato (1960) listed the standard Eh values for some sulfide minerals as follows:

1. $Cu_2S = CuS + Cu^{2+} + 2e.$ Eh = 0.530 volts

2. $CuS = Cu^{2+} + S + 2e.$ Eh = 0.591 volts

3. $PbS = Pb^{2+} + S + 2e.$ Eh = 0.354 volts

4. $FeS_2 = Fe^{2+} + S_2 + 2e.$ Eh = 0.757 volts

5. $ZnS = Zn^{2+} + S + 2e.$ Eh = 0.265 volts

From such values as these it can be predicted that in a mixture of chalcopyrite and pyrite, chalcopyrite (Eh = 0.52 volts) should dissolve in perference to pyrite, and in a mixture of sphalerite (ZnS) and pyrite, sphalerite should dissolve in preference to pyrite. This is what Mehta and Murr (1982) found. Moreover, they showed that the respective rates of chalcopyrite and sphalerite dissolution were faster in the presence than in the absence of pyrite. When they inoculated the mineral mixtures with the mesophilic T. ferrooxidans at 30 degrees Celsius or with a thermophilic, acidophilic iron oxidizer at 55 degrees Celsius, they increased the rates of chalcopyrite and sphalerite leaching further, the greater increase being at 55 degrees Celsius. Berry et al., 1978; Mehta and Murr, 1982 attributed the bacterial effect to removal of a passivating layer of sulfur by oxidizing it to sulfuric acid on the mineral surface. Murr and Mehta (1983) further strengthened the concept of a galvanic contribution to mixed- sulfide- mineral leaching by showing that in the sphalerite/pyrite mixture, no ferric iron was formed which might otherwise have contributed to enhanced sphalerite dissolution. The bacteria were seen to contribute to catalytic oxidation of anodic sphalerite and cathodic pyrite, but iron release remained small. Berry et al. (1978) and Murr and Mehta (1983) also provided visual evidence of a galvanic effect for a chalcopyrite/pyrite couple in the form of scanning electron micrographs.

chalcopyrite/pyrite couple in the form of scanning electron micrographs.

Galvanic effects may help to explain difficulties which can be encountered in the leaching of complex ores.

4.1.c Bioleaching of Uranium Ores

Bioleaching of uranium ores with acidophilic, iron-oxidizing bacteria is being commercially exploited. The process involves heap-, dump-, and in-place leaching. Canada is one of the users of this process. See section 5.1.1 for more details.

4.1.d Bioleaching of Metal Oxide Ores

4.1.d.1 Metal Oxides From Oxidized Portions of Sulfide Ore Bodies

Cuprous and cupric oxides as well as carbonates and sulfates and to a more limited extent silicates, formed in oxidizing zones of sulfide ore bodies, are leachable by the dilute sulfuric acid formed by acidophilic iron bacteria in the oxidation of pyrite, FeII or sulfur. This activity is commercially exploited in an empirical manner in the bioleaching of ore containing both sulfide mineralization and secondary, oxidized mineralization.

4.1.d.2 Metal Oxides, Carbonates, and Silicates, Not Secondarily Formed in Oxidizing Zones of Sulfide Ore Bodies

Metal oxides like those of Cr (crocoite, PbCrO), Fe [hematite (Fe_2I_3), goethite, ($Fe_2O_3.2H_2O$), ferric oxhydroxide (FeOOH)] and Mn [pyrolusite or birnessite (MnO_2), etc.] may be bioleached by reduction to a lower oxidation state with an organic electron donor such as glucose (Lebedeva and Lyalikova, 1979; Munch and Ottow 1980; Ehrlich, 1980). Senarmontite (Sb_2O_3) formed chemically, or biologically from stibnite (Sb_2S_3) by T. ferrooxidans, may be oxidized to Sb_2O_5 by Stibiobacter senarmontii (Lyalikokva, 1974).

Metal oxides like cassiterite (SnO_2), and especially varmaloffite ($SnO_2.2H_2O$), can be leached by oxalic and citric acids produced by fungi (Teh et al., 1981). However, the best recoveries so far obtained have only been on the order of 10% of the ore content.

Aluminum may be leached from clays (aluminosilicates), including red mud, with biogenic acid produced by T. ferrooxidans oxidizing FeII (Zimmerley et al., 1958; Szolnoki, 1975), by organic acids produced by bacteria such as Bacillus mucilaginus (Andreev et al., 1979), or B. ciculans (Groudev and Genchev, 1978). Mehta et al. (1978) used Penicillium simplicissimus to leach aluminum from rock particles with a 20% yield. The present difficulties with bioleaching of aluminum on a commercial scale are the limitation in extraction and its slow rate.

Extraction of titanium is claimed in the patent of Zimmerley et al.(1958) to occur during bioleaching of sulfide ores. Bioleaching of vanadium from vanadiferous uranium ore has been patented by Goren (1966). The process uses T. ferrooxidans.

Metal carbonates like rhodochrosite, malachite, siderite, etc. can be leached by acid produced biogenically from sulfur by T. thiooxidans or from sugar by heterotrophic bacteria or fungi.

The economics of any of the above processes, especially those employing heterotrophs, is very dependent on the cost of producing the acid needed for leaching. Production of organic acids is generally much more expensive than production of inorganic acids such as sulfuric acid.

4.1.e Bioleaching of Gold- and Silver-Containing Ores

4.1.e.1 Bioleaching of Gold

Currently a wide-spread interest exists in the mining industry in the application of biohydrometallurgy to the extraction of precious metals (e.g., gold and silver) from ores to replace cyanidation or other complexing method or to supplement it.

Gold has been extracted from laterites and clays by unidentified complexing agents produced by Agrobacterium tumefaciens and Serratia marcescens (Pares, 1964a,b). It has also been extracted with amino acids (specifically histidine, asparagine, serine, aspartic acid, alanine, and glycine) as well as proteins of microbial origin, isolated, for instance, from the culture fluid of Bacillus mesentericus niger 12 by Korobushkina et al (1976). The authors concluded that anionic complexes were produced apparently by formation of Au-N bonds under alkaline conditions. They inferred this from infrared analyses of some gold complexes. Korobushkina et al (1983) modified the interpretation of gold reactions with amino acids. They concluded that in alkaline media, an excess of glycine, alanine, valine or phenylalanine formed coordination complexes with AuI via amino-acid carboxyl groups, histidine via coordination complexes involving amino- and imidazol nitrogens, and cysteine via its sulfhydryl group. Additional functional groups in serine, phenylalanine and tryptophane did not participate in gold complex formation. The dual amino groups in lysine and arginine were reported to increase their complexing capacity. AuI was reported to form a most stable complex with cysteine. Histidine and then asparagine were next in effectiveness. Stability of AuI-amino acid complexes was very redox-potential-dependent.

Korobushkina et al. (1983) reported data from Mineev (1976) indicating that 2.15 mg of gold per liter were dissolved by a strain of Bacillus megaterium in 110 days at pH 8 to 8.5. Pretreatment of metallic gold with Na_2O_2 increased its reactivity with complexing agents.

Thiobacillus ferrooxidans was shown by Lyalikova and Mokeicheva (1969) to solulbilize up to 520 mg of gold per liter in shake cultures from 5 g of gravity concentrate (150 g gold per tonne) containing sulfide mineralization. The leaching was done in 200 ml of Leathen's medium at pH 3.5 over 3 weeks. T. ferrooxidans and other Thiobacillus species (Thiobacillus Y; T. denitrificans at pH 7.8) were also active in solubilizing gold from antimonite and galena. The solubilized gold may have been in the form of polythionate complexes. These authors also isolated a strain of the heterotroph Bacillus alvei from the Zodsh gold deposit which solubilized up to 560 ug of gold per liter from the gravity concentrate in a peptone-succinate medium These gold yields were 5 to 7 times less than obtained by Pares (1964a,b) from laterites.

4.1.e.2 Beneficiation of Gold-Containing Ores By Microbial Leaching

Gold recovery from sulfide ores by cyanidation is inefficient because the sulfide consumes cyanide which then is unavailble for complexing of gold. Elimination of these sulfides (e.g., pyrite, arsenopyrite, stibnite, etc.) prior to cyanidation to extract gold is of obvious benefit. T. ferrooxidans oxidizes such sulfides and has been suggested as an agent to beneficiate gold-containing sulfide ores (Kamalov et al., 1973; Pol'kin et al., 1977; Solozhenkin et al., 1982; Livsey-Goldblatt et al., 1983). Livsey-Goldblatt et al.(1983) examined a bacterial beneficiation process of an arsenopyrite/pyrite-, gold-containing ore using run-of-the-mine ore and ore concentrate and compared it to a conventional flotation-roast process. In all cases, gold was recovered from the beneficiated ore by cyanidation. The authors found gold recovery by microbial beneficiation of ore concentrate but not run-of-the-mine ore competitive with conventional flotation-roast beneficiation (7,701 Rands for concentrate versus 8,825 Rands for flotation-roast and 10,391 Rands for run-of-the-mine).

4.1.e.3 Bioleaching of Silver

Relatively little work has been done on bacterial leaching of silver from ores. Most studies have yielded unfavorable results. One special problem which has been generally perceived is the toxicity of silver for microorganisms. Depending on the experimental conditions, silver toxicity levels have been reported as 0.1 ppb (Norris and Kelly, 1978), 0.1-0.5 ppm (Hoffman and Hendrix, 1976), and 50 ppm (Tuovinen et al, 1971) for T. ferrooxidans. Toxicity is due to the interference of Ag^+ with electron transport, ATP synthesis, phosphate transport, etc. of microorganisms (Bragg and Rainnie, 1973; Schreurs and Rosenberg, 1982).

Insofar as leaching of Ag_2S by T. ferrooxidans in a ferrous sulfate-containing mineral-salts medium is concerned, Norris and Kelly (1978) reported finding 0.14 ppm of Ag^+ in solution after 200 h. Pooley (1982) reported the precipitation of Ag_2S on the walls of cells in a mixed culture of T. ferrooxidans and T. thiooxidans in a leaching experiment involving silver-containing sulfide minerals. He also found such silver sulfide precipitate to form on the cell mixture in a medium with elemental sulfur and silver nitrate, but not in a medium containing ferrous sulfate and silver nitrate but no sulfur. He found that this precipitated silver sulfide did not inhibit the activity of the bacteria. He also found that this silver sulfide was recoverable by cyanidation. He suggested that this phenomenon might be exploitable in the recovery of silver from silver-containing sulfide ores and might even be applicable to the recovery of gold.

4.1.f Metal Recovery From Industrial Wastes Such As Fly-Ash, Slags, Sludge, Tailings From Galvanic Processes, and Flotation Residues

Investigations into the recovery of valuable metals such as Cu, Ni, Zn, Cr, Pb, Cd, As, and V from industrial wastes by bacterial leaching with thiobacilli and heterotrophs are currently underway in Germany (Ebner, 1978; Bosecker, 1983; Schaefer, 1983). Metal recoveries ranging from 30 to 100% have been reported depending on the nature of the material and the microbial process employed. Separation of aluminum from red mud has also been reported (Szolnoki, 1975; 1977).

Metal recovery from such wastes facilitates their disposal by minimizing environmental pollution by toxic metals from "spontaneous" leaching. It also results in products which at the least may help to pay for the waste disposal process.

4.1.g Sulfur Recovery From Acid Mine-Drainage Not Employed in Dump-, Heap-, or In-Place Leach Cycles

The feasibility of converting sulfate in acid mine-drainage from sulfide ore deposits and hydrometallurgical ore extraction to sulfur by microbiological means has been demonstrated (Cork and Cusanovich, 1978). The process employs sulfate-reducing bacteria such as Desulfovibrio desulfuricans to reduce sulfate to sulfide using lactate as electron donor. The sulfide is converted to elemental sulfur by the green photosynthetic bacterium *Chlorobium thiosulfatophilum* or *C. vinosum*. The process is anaerobic and requires two reactions, one for sulfide production from sulfate and the other for sulfur production from sulfide. The process can be run in a continuous mode and is applicable not only to acid mine-drainage from sulfide ore deposits and ore extraction processes but also from coal deposits, etc. (Cork and Cusanovich, 1979; Cork, 1982; Cork et al., 1983).

B.C. Research of Vancouver, British Columbia, claims to have modified their process for leaching copper sulfide ores patented by McElroy and Duncan (1974) such that the sulfide-sulfur in the ore is converted to elemental sulfur instead of sulfate (Short and Parkinson, 1983). This is accomplished by adding soluble copper and thiosulfate in addition to silver to the reaction mixture. The chemistry by which the elemental sulfur is formed has not been worked out as yet.

4.1.h Microbial Destruction of Cyanide in Waste Streams

Cyanide in the environment represents a biological hazard because of its toxicity. Since cyanide is used by the minerals industry as suppressor in froth flotation for the separation of mixtures of certain sulfide minerals and in the recovery of gold and silver by cyanidation, it may be present in waste streams from such processes (Huiatt et al., 1983). The possibility of eliminating this cyanide microbiologically exists.

At least three enzyme systems are known in microorganisms which can transform cyanide. One of these is nitrogenase (prokaryotes only). The second is rhodanese (prokaryotes and eukaryotes). The third is one which converts cyanide to ammonia and carbon dioxide.

Nitrogenase is involved in nitrogen-fixation in which dinitrogen (N_2) is reduced to ammonia (NH_3). The same enzyme can, however, catalyze reduction of cyanide to methane and ammonia (Hardy and Knight, 1967). Because the enzyme is oxygen sensitive, it is not the preferred choice for industrial treatment of cyanide-containing waste streams.

Rhodanese is an enzyme which can catalyze a reaction between cyanide and thiosulfate leading to the formation of thiocyanate and sulfite, both of which are less toxic than cynide,

$$CN^- + S_2O_3{}^{2-} = SCN^- + SO_3{}^{2-}.$$

Thiosulfonate, persulfide, polysulfide, ethanesulfonate, and thiotaurin can substitute for thiosulfate in this reaction (see Knowles, 1976). Sulfite is metabolizable and can be converted to sulfate by a variety of aerobic microorganisms. The feasibility of cyanide destruction in a continuous system has been explored by Atkinson (1975). He used a thermophilic bacterium, Bacillus stearothermophilus, and found that at 50 degrees Celsius, it detoxified 14 to 125 g of NaCN per liter per hour when in 50 ml of bacterial suspension (8 mg dry weight per ml) in a stirred 65-ml ultrafiltration cell fitted with an 0.22 um-filter overlayed with a microfiber prefilter. The suspension medium consisted of 50 or 5 mM NaCN, 100 or 10 mM sodium thiosulfate, 5 mM magnesium chloride, 1 mM EDTA, and 0.1 mM phenylmethylsulfonylfluoride. Atkinson (1975) was able to isolate a strain of B. stearothermophilus resistant to 10^{-3} M NaCN. It contained 5 to 6 times the amount of rhodanese found in the parent strain.

An enzyme system also appears to exist which can convert cyanide to CO_2 and ammonia with the carbon of cyanide forming formate as an intermediate. Fungi like Fusarium solani seem to have this capacity (Shimizu and Taguchi, 1969). An industrial-scale utilization of this process for treating cyanide-containing wastes has been examined by Shimizu et al (1970).

Some microorganisms can assimilate cyanide, i.e., they can use it as a carbon and nitrogen source or a nitrogen source (see Knowles, 1976 for a review). Such organisms could be useful in the removal of cyanide from waste streams. Similarly, some autotrophs and *Pseudomonas nonliquefaciens* have been found to use thiocyante as a carbon and nitrogen source or a nitrogen source (see Knowles, 1976 for a review). Such organisms would be useful in removing the thiocyante from solution formed by the rhodanese reaction.

Cyanides and cyanide complexes of some base metals have been decomposed microbiologically in sewage treatment in some instances (for brief reviews see Knowles, 1976; Huiatt, 1983).

4.1.i Microbial Desulfurization of Coal

Bituminous coal, and to a very small extent anthracite coal, contain a significant amount of sulfur, including organic and inorganic forms. The organic sulfur is in the form of -R-S-S-R-, -R-S-R-, or as organically bound sulfate ($R-O-SO_3$) (Dugan and Apel, 1978). The inorganic sulfur occurs as pyrite and sulfate (e.g., gypsum). On the average, organic and inorganic sulfur constitute up to 7% of the coal in the USA. In many coals the organic sulfur is dominant, but in some coals from the eastern USA, pyritic sulfur is dominant, amounting to as much as 4% at times (Dugan and Apel, 1978).

4.1.i.1 Microbial Pyrite Removal From Coal

The pyrite of coal can be removed microbiologically if the coal is first pulverized and subjected to oxidative attack by acidophilic, iron- and sulfur-oxidizing bacteria. Dugan and Apel

(1978) were able to remove 97% of the pyritic sulfur from a coal sample containing 4.6% total sulfur, of which 3.1% was pyritic and 0.1% was sulfate. The coal was in a slurry with a mixed culture of acidophiles. They found enrichment of the slurry with 0.3% ammonium sulfate and an initial pH of 2.0 to 2.5 stimulated bacterial activity. By comparison a purely chemical oxidation process, using 1 M aqueous ferric sulfate or ferric chloride removed 88 to 98% of the pyritic sulfur but transformed it into elemental sulfur and thus required subsequent toluene extraction to remove the elemental sulfur (Meyers et al., 1972).

Olson et al. (1980) have reported on the kinetics of microbial pyrite oxidation of pulverized coal, relating pulp density (PD) to reaction velocity (V) by a modified Monod equation

$$V = V_m(PD)/(K) + (PD),$$

where V_m is maximal velocity and K is a constant. They also determined the reaction order by application of the van't Hoff equation,

$$V = kC^n,$$

where V is the rate of pyrite oxidation, C is the initial pyrite concentration, and k and n are constants. Furthermore, they studied the kinetic effects of particle size and oxygen mass transfer.

Murr and Mehta (1982) have reported that thermophilic, acidophiles can leach pyritic sulfur from coal at a somewhat faster rate than mesophilic acidophiles. Kargi (1982) has reported that an external CO_2 supply stimulates leaching of pyritic coal by T. ferrooxidans at a high cell concentration. Kargi and Robinson (1982) found that the thermophilic Sulfolobus acidocaldarius can remove inorganic sulfur from coal, especially in the absence of organic supplements such as yeast extract and peptone. In ten days, they were able to remove 96% of the inorganic sulfur from a coal sample that contained 4% total sulfur of which 2.1% was inorganic with the coal in a 5% slurry.

Pooley and Atkins (1983) have reported that very short-term attack of pyrite in coal by bacteria (T. ferrooxidans) enables the separation of the pyrite by flotation. The froth-flotation response of the weathered pyrite appears not to be due to mere cell attachment.

No really successful processes for bioextraction of organic sulfur compounds from coal have been reported. Some controversy exists as to whether coal can serve as a major nutrient to microorganisms. For example, Koburger (1964) reported only limited growth of Escherichia freundii and Pseudomonas rathonis on coal after treatment with hydrogen peroxide to eliminate growth inhibitors. Cohen and Gabriele (1982), Trueper (1983), and Lingens (1983), on the other hand, reported growth on and utilization of coal by microorganisms, chiefly fungi. Some of these contradictory findings may be attributable to the differences in rank of the coals used. Lignite can be expected to be a more likely source of microbial nutrients because it is a less mature coal chemically than bituminous coal or anthracite. Nevertheless, the newer reports of fungi which grow in coal slurries may present a point of departure for developing strains which may specifically attack organic sulfur compounds in coal when supplemented with other essential nutrients (see section 6.1). At present, commercially attractive microbial desulfurization of coal applies only to coal in which inorgnic sulfur is the chief sulfur constituent.

4.1.j Metal Removal From Dilute Waste Streams By Microbial Means

Current research and intiatives on this topic are included in section 3.2.2.

4.1.k Microbial Rust Removal and Descaling

4.1.k.1 Rust Removal by Bacterial FeIII Oxide Reduction

Although this problem has not been specifically addressed by microbiologists up to now, it is technically feasible. Various types of bacteria have been detected in soil, freshwater, and marine environments which can utilize FeIII as terminal electron acceptor in their respiratory process (For a summary see Ehrlich, 1981; also Munch and Ottow, 1980; 1982; Ottow, 1982; Jones et al., 1983). These bacteria reduce FeIII to FeII with a suitable electron donor such as glucose (but see Obuekwe et al., 1981). They can attack FeIII in the form of Fe^{3+}, FeOOH, goethite, hematite, etc., but not all with equal ease. Rust is a form of ferric oxide. It must be understood that microbial rust removal by this means does not protect against further corrosion but may actually enhance it (Obuekwe et al., 1981a). This effect is best understood in the sense that by removing the rust, the iron or steel surface loses its passivation layer of ferric oxide.

4.1.k.2 Rust Removal by Siderophore Treatment

Siderophores (hydroxamate- or catechol-based) have a high affinity for ferric iron ($K_a = 10^{38}$ to 10^{50}) but a low affinity for FeII ($K_a = 10^8$). These compounds are produced by many prokaryotic and eukaryotic microorganisms which live at neutral pH. However, the quantity of siderophore produced by a wild-type culture is limited. For industrial exploitation, siderophore production would have to be enhanced (by gentic engineering). Concentrated solutions of siderophores should be very effective in rust removal. However, as in the case of rust removal by FeIII-reducing bacteria, such removal will not protect against renewed corrosion.

4.1.1 Biohydrometallurgy and the Production of Metal Powders

It has been suggested that metal powders can be produced from pregnant solutions generated by bioleaching of metallic ores, such as copper ores (Das et al., 1981). Copper powder may be produced by cementation, i.e., by reaction of the Cu^{+2} in pregnant solution with scrap iron. Reaction with iron nails, iron wire scrap, punchings, etc. are said to give copper powders of good flowability and a purity as great as 99.8% after drying in a hydrogen atmosphere (Das et al., 1981).

Another method for producing copper powders is the reduction of cuprous cyanide prepared from preganant solution by dispersal of the cyanide in paraffin oil and reducing it with hydrogen at 300 C. The mineral oil can be removed from the copper powder product by washing with appropiate solvents (Das et al., 1981).

A third method for producing metal powders (e.g., Cu or Ni powders) is by pressure reduction in titanium- or stainless steel-lined autoclaves at 25 Atm and 200 C (Das et al., 1981).

The metal powders can meet at least part of the demands of the powder metallurgical branch of the metals industry.

4.2 MECHANISTIC STUDIES IN MICROBIAL PHYSIOLOGY

Studies into the biochemistry of FeII oxidations and concurrent generation of useful energy by acidophilic bacteria is continuing (see review by Ingledew, 1982). Studies on the bacterial oxidations of FeII at neutral pH is ongoing in the laboratories of H. Hanert (Technical Univeristy of Braunschweig, West Germany) and W.C. Ghiorse (Department of Microbilogy, Cornell University, Ithaca, NY, USA). The mechanisms of bacterial oxidation of MnII continue to be investigated in the laboratory of H.L. Ehrlich (Biology Department, Rensselaer Polytechnic Institute, Troy, NY, USA) and Catherine E. Douka Department of Soil Science, Nuclear Research Center "Demokritos", Aghia Paraskevi Attikis, Athens, Greece). Dissimilatory sulfate reduction continues to be studied in the laboratory of H.D. Peck, Jr. (Department of Biochemistry, University of Georgia, Athens, GA, USA), in the laboratory of J. Le Gall (Laboratoire de Chimie Bacterienne, and in the laboratory of N. Pfennig (Institute fuer Mikrobiologie der Gesellschaft fuer Strahlen- und Umweltforschung mbH Muenchen in Goettingen, West Germany). Investigations into microbial oxidation of inorganic elements like As and Sb are being undertaken at the Academy of Sciences of the USSR in Moscow (N.N. Lyalikova and associates).

Investigations into the use of reducible transition metals as terminal electron acceptors is currently being reported with respect to FeIII from the laboratory of J.C.G. Ottow (University of Hohenheim, West Germany) and the laboratory of D.W.S Westlake at the University of Alberta, in Edmonton, Alberta, Canada). Work with respect to bacterial reduction of Mn(IV) is being reported from the laboratory of B.J. Ralph (University of New South Wales, Sydney, Australia) and A.D. Agate (MACS Research Institute, Pune, India). Work with respect to bacterial reduction of Cr(VI) was recently investigated by Romanenko and Koren'kov (Academy of Sciences, Moscow, USSR) and in the laboratory of H.L. Ehrlich (Department of Biology, Rensselaer Polytechnic Institute, Troy, NY, USA). The reduction of Mo(VI) to Mo(IV) by Sulfolobus, using elemental sulfur as reductant, was recently reported by C.L. and J.A. Brierley (Advanced Mineral Technologies, Inc., Socorro, NM, USA).

A more complete understanding of the enzymatic interactions with metals may permit the design of systems in the future which enzymes rather than cells are used in metal extraction by precipitation. Purified enzymes may produce faster reactions than the cells which produced the enzymes.

4.3 GENETIC IMPROVEMENT AND STRAIN SELECTION

Microbial metal extraction and concentration processes can be improved by (1) increasing the rates of reaction, which may be achieved by increasing the concentration of the enzyme system for metal extraction, or the metal-binding component for metal concentration; (2) accelerating the growth rate of microbes whose action depends on cell multiplication; (3) eliminating interfering biochemical reactions, and (4) increasing the tolerance of active microorganisms for toxic metals which they are to extract or concentrate. Improvement in each of these traits may be achieved by appropriate genetic manipulation. Possible methods of manipulation are (1) selection of spontaneous mutants in a wild-type population of appropriate strains of microorganisms, which is possible if suitable selective growth conditions are used; (2) selection of induced mutants in a wild-type population, with induction being achieved with ultraviolet or X-radiation or with mutagenic chemicals; (3) chromosomal recombination (in prokaryotes including transformation, transduction, and conjugation and in eukaryotes, sexual reproduction); (4) plasmid transfer by transformation, transfection, transduction, or conjugation from a bacterial strain not useful for a particular metal-extraction or concentration process to a strain that will become useful by this genetic modification (5) recombinant DNA technique, the simplest approach being to construct a transferable plasmid with the desired characteristics and introducing it into a suitable wild-type strain; and (6) gene or plasmid amplification.

4.4 SCALE-UP PROBLEMS

4.4.1 General Comments Concerning the Practice of Bioleaching

Scale-up of bioleaching operations does not follow known standard procedures because bioleaching is a rather broad concept. As far as engineering design is concerned, the operation can vary from in-place, heap, or dump operations to controlled, i.e., batch or continuous operations in tanks containing ore concentrates.

A detailed experimental program specific for a metal to establish the best choice of organism or mixture of organisms to be used and to evaluate key parameters of the leaching process is essential for the development of a large-scale operation. General criteria and several engineering considerations can, however, be used for approximating scale-up of a proposed process before its full evaluation.

Although no standard procedures for bioleaching have yet been developed, there are some general types of processes with characteristics best suited for specific circumstances such as the nature of the raw material to be treated, operation and quality restrictions, etc. Among the process types, four will be briefly discussed, namely dump-, heap- and in-place bioleaching, batch bioleaching, continuous bioleaching, and semi-batch/semi-continuous bioleaching.

4.4.1.1 Dump-, Heap-, and In-Place Bioleaching

In-situ bioleaching has been successfully used to extract copper from low-grade sulfide minerals and tailings with copper contents of less that 0.4% (Sheffer and Evans, 1968). It involves circulation of leach solution through a broken orebody.

For dump- and heap- leaching, low-grade ore is deposited in piles on top of an impermeable substratum (heaps may be higher than 200 m). The leaching solution is applied at the top and allowed to percolate through the ore to be collected at the bottom. In general, leach dumps make use of natural slopes for the recovery of the pregnant solution which contains around 2 g/L of copper when the ore is leached, and which is processed further to recover the metal.

The advantages of this kind of leaching are mainly its simplicity and low cost. It is already operated on an industrial scale. This type of process does not follow the generalized block diagram (Fig. 5) and makes do without most (if not all) of the unit operations needed for the other processes. The disadvantages are the lack of controllability of the process which can lead to problems like slow rate of reaction, excessive inhibitor levels, microbial washout, selection of unsuitable microbial mutants, and environmental factors such as temperature, moisture content, pH, particle size, surface area, nutrient supply, and oxygen transfer.

4.4.1.2 Batch Process

For bioleaching by a batch process, a tank is essential. It is a closed system. As the bioleaching process proceeds in the tank, the relative metal concentration in the ore and in the pregnant solution changes with time. The advantages of this type of process include flexibility, adaptability, relatively simple operation, and more control over the active organisms. Distinct disadvantages are low productivity due to long downtime, large concentrations of inhibitors (the metals) building up toward the end of the leach, and the difficulty of controlling the reproducibility of the metal yield from one batch to the next.

Scale-up problems will be encountered if the degree of homogeneity kept on a laboratory scale is difficult to obtain on a larger scale. Oxygen and heat transfer may be more difficult to control. Dead volumes present a common scale-up difficulty in this type of process.

4.4.1.3 Continuous Process

Ideally, it should be possible to maintain bacterial activity in a continuous bioleaching process for prolonged periods of time. Furthermore, it should be possible to achieve a steady state and maintain it such that the culture environment (e.g., nutrient and relative metal concentrations in the pregnant liquor and ore) and all activities remain constant. An example of this is presented by McElroy and Brynesteyn (1978).

The advantages of continuous bioleaching are its high productivity level and the removal of inhibitors and possible on-line feedback for quality control.

4.4.1.4 Semi-Batch/Semi-Continuous

A process that involves time periods of batch operation combined with time periods of addition of fresh feed and/or drainage of products and by-products is semi- batch/semi-continuous. Fresh feed could be either addition of new mineral concentrate and/or recycled material as well as inoculum (when necessary) and water, oxygen, and nutrients. The characteristics for these kinds of processes are a combination of those for batch and continuous processes. A possible new design of a bioleaching operation in spouted beds is currently being investigated (Morgan, 1983; see also Mathur and Epstein, 1974; Mathur and Gishler, 1955; and Manurung, 1964 for further discussion and illustration of the principles of spouted bed operation).

4.4.2 Leaching of Low-Grade Ore

Methods of metal extraction from low-grade ore by heap-, dump-, or in-place bioleaching provide an acceptable degree of recovery in reasonable time and at low cost.

When bioleaching is applied to ore concentrates, laboratory scale data will only indicate which factors may be important to control. It is necessary to carry out experiments on larger scales to determine how this influences final extraction, mean daily extraction, contact time, time needed to regenerate the leaching solution, etc.

Because these final results depend on the particle-size distribution of the ore and on the quality of ore to be processed, it is not enough to obtain all the data only from bench-scale experiment to scale up to a desired industrial size. Once the need to build a semi-industrial installation arises, it is necessary to find a methodology to scale up pilot plant data. With such methodology for a given type of ore it will be possible to scale up directly from laboratory and pilot plant tests to industrial installations.

4.4.2.1 Background Theory

To better understand the fundamentals of a scale-up procedure for bioleaching of low-grade ores, we will start with basic concepts.

As Ilie et al. (1983) have discussed, the process of bioleaching of a nonferrous sulfide mineral can be considered as a heterogeneous reaction which is carried out in a diffusion controlled regime. Under these conditions, the kinetics of the process can be described by the equation:

$$V = \frac{D}{R} A(C1 - C2) \quad (1)$$

where:

V is the leaching rate,

D is the diffusion coefficient,

R is the total resistance,

A is the total surface area,

C_1 is the saturation concentration,

C_2 is the instantaneous concentration.

In this case it will be assumed that the quantity (DA/R) is constant and denoted by K. Thus equation (1) becomes

$$V = K\ (C_1 - C_2) = K\ \Delta\ C \quad (2)$$

Because rate (V) may be expressed in terms of change in concentration with respect to time, we can write:

$$V = dC/dt \quad (3)$$

Experimental data indicate that in bioleaching of minerals, microorganisms play a partially catalytic role. A general equation for the solubilization of the nonferrous metal sulfide (MS) in an acidic ferric iron solution is:

$$MS + 2Fe^{3+} \text{------>} M^{2+} + 2Fe^{3+} + S^{o} \quad (I)$$

Independent of the sulfide selected, the first stage of oxidation of the sulfide involves an electron transfer from S^{2-}

to Fe^{3+} with the formation of S^o. Although formation by ferric iron oxidation of a base metal sulfate according to the reaction:

$$MS + 8Fe^{3+} + 4H_2O \text{------>} M^{2+} + SO_4{}^{2-} + 8H^+ + 8Fe^{2+} \qquad (II)$$

is negligible, the bacterial oxidation of elemental sulfur formed in reaction (I) is significant,

$$S^o + 3/2\ O_2 + H_2O \text{------>} H_2SO_4 \qquad (III)$$

According to this chemistry, the rate of leaching will depend on the available exposed surface concentration of the mineral components at the solid-liquid interface, the concentration of the oxidizing agent, and the presence of a bacterial catalyst. It is practically independent of the diffusion of ions in the interior of the solid phase. Consequently it is necessary for scale-up to keep the factor K constant.

From the previously stated consideration, keeping conditions constant, we can write as follows:

$$\frac{q(p)}{Ap} = \frac{q(1)}{a1} \qquad (4)$$

in which:

q(p) is the quantity of solution in the pilot stage;

Ap is the total surface of mineral used in the pilot stage;

q(1) is the total quantity of solution used in the laboratory;

al is the total surface of mineral used in the laboratory.

We should mention that these conditions are valid only for the initial stages of the process and that the relationships express absolute values for the chosen leaching process. The conditions will not necessarily remain constant over an extended period of reaction. In the subsequent stages, the leaching process is a lot more complex and the rate of leaching involves numerous, simultaneous and complicated relations with components of the process other than the surface area. The longer the duration of the leaching, the better thei relationships can be appreciated. However, it is more convenient to express the kinetics of the process based on a single limiting parameter, while keeping other parameters constant. In the case of bioleaching, the limiting parameter is considered to be the surficial metal concentration.

4.4.2.2 Determination of the Surficial Metal Concentration According to Ilie et al. (1983)

For the determination of the surficial metal concentration, a metal is arbitrarily assumed to be uniformly distributed throughout the mass of the mineral. For the volume of a stratified surface of mean thickness (a) and for a species of granulosity with mean diameter (d), the concentration of metal in the grains can be expressed by the following equation:

$$q'_m = G_g a d^2 \frac{C}{B\,100} \quad \text{(unit of weight)} \qquad (5)$$

where:

q'_m is the quantity of metal available per grain of diameter d;

G_g is the weight of a grain of diameter d;

a is the mean thickness of the available layer for bioleaching;

d is the diameter per grain;

B is the total volume per grain;

C is the mean content of available metal per grain in per cent.

For the integral quantity of species "i" we can write:

$$Q'_{M(i)}=6\ \frac{G(i)a}{d(i)} \cdot \frac{C}{100} \quad \text{(unit of weight)} \qquad (6)$$

(Note: in the article by Ilie et al. (1983), equations (6) and (7)are written differently. We believe that this difference is due to a typographical error where G should be 6).

In equation (6) above:

$Q'_{M(i)}$ is the weight of the superficial metal of species "i";

a is the mean surperficial thickness;

d(i) is the diameter of class "i";

G(i) is the total quantity of species "i";

C is the mean content of available metal in percent.

The equation for the overall quantity becomes:

$$Q'_M=6aG_T\ \frac{C}{(100)}\ 2\ \sum_{i=1}^{n}\ \frac{1}{di}\ Xi \qquad (7)$$

where:

Q'_M is the quantity of surficial metal;

a,C same notations as in equation (6);

G_T total quantity of ore;

di mean dimension (diameter) of a grain of species "i" in cm;

Xi percent of species "i".

4.4.2.3 Determination of the Leaching Progress as a Function of the Available Surficial Metal Concentration and the Quantityof Solution

Leaching is assumed to advance from the surface of the mineral grains towards their center. Expressing leaching progress as a function of available surficial metal sulfide concentration and maintaining the same conditions as in the laboratory test:

$$\frac{q(1)}{Q'_M(1)} = \frac{q(L)}{Q'_M(L)} = k \qquad (8)$$

where:

$q(s)$ is the quantity of solution used;

$Q'_M(s)$ is the quantity of surface metal for:

s=1, laboratory or pilot plant conditions;

s=L, semi-industrial scale conditions;

s=Lp, industrial scale conditions.

Assuming all other physicochemical and biological parameters to be identical for the two scales, the unknown element q(L) could be expressed from the previous equations:

$$q(L) = q(1) \frac{Q'_M(L)}{Q'_M(1)} \quad \text{(in cubic meters or liters)} \qquad (9)$$

The quantity of solution q(L) determined from this equation is thus the total quantity needed to insure the same rate of leaching progress for the scaled-up stage as it was for the previous stage.

In practice, however, it is not possible to disregard that for quantities of ore on the order of thousands of cubic meters, the mean daily extraction is smaller than from a small quantity of ore. Thus a longer time will be required to extract a given amount of metal. The solid/liquid ratio obtained from the above equation is very small compared with that reported in the literature. Keeping all these limitations in mind, we can write:

$$V = v \frac{q(L)}{q(Lp)} \qquad (10)$$

from which, substituting for q(L), we obtain:

$$V = v \frac{q(1)\ Q'_M(L)}{q(Lp)\ Q'_M(1)} \qquad (11)$$

where:

V is the scaled-up, expected progress rate in g/L/day;

v is the smaller scale progress rate in g/L/day;

$q(Lp)$ is the quantity of solution used in practice in cubic meters.

In case of a very high metal sulfide content, a factor Y, which represents (as a percentage) the "contacted" mineral sulfide zone, is introduced as a function of the solid/liquid ratio denoted by r:

$$\frac{GT}{q(Lp)} = r \frac{Y}{100} \qquad (12)$$

From the equation for the rate of leaching progress, and at the same time replacing the expression for the surface metal sulfide in its explicit form, the following relation will result:

$$V = var \frac{Y}{100} \frac{q(1)}{Q'_M(1)} \frac{C}{100}\, 2 \sum_{i=1}^{n} \frac{1}{d_i} \quad (g/L/day) \qquad (13)$$

From this equation it can be seen that the change of the rate of leaching progress does not depend directly on the quantity of liquid used but only on the intermediate solid/liquid ratio previously considered. Thus for this to correlate with the rate of progress, the quantity of liquid will be expressed as:

$$q(Lp) = \frac{G_T}{r} \tag{14}$$

where the notation is the same as for equations (7), (9) and (12).

Calculations for a scale-up for a semi-industrial stage of about 50,000 tons as developed by Ilie et al. (1983) will now be shown, starting from the pilot plant stage data shown in Table 14 and the granulosity analyses shown in Table 15.

Pilot stage:

The quantity of metal on the surface is calculated from equation (7) and shown to have a value of $Q'_{M(1)} = 0.685$ kg. Using the same equation, the quantity $Q'_{M(L)} = 1,313.342$ kg is obtained.

From the data for the daily rate of progress, we calculate that for the pilot plant stage, the mean rate is 1.914 g/L/day (0.0063 g/L/day from Ilie et al., 1983) for a solid/liquid ratio of 1.4:1 (see Table 14).

The total quantity of solution for the semi-industrial stage is caculated to be 20,500 m^3 from equation (9), thus giving a ratio of $r = 2.44$. This theoretical ratio is, however, very small compared with the values reported in the literature (real data), which are of the order of hundreds. We calculated the variation of the rate of enrichment, the quantity of solution, and its corresponding value of r covering the range from 25 to

Table 14. Pilot plant data

Specification	Data from Ilie et al. (1983)
Total quantity of ore, tonnes	15
Available metal content (%)	0.21
Total volume of solution used (m^3/day)	10.7
Solid/liquid ratio (t/m^3/day)	1.4/1.0
Total duration of leaching (days)	319
Mean daily rate of leaching (g/L/day)	0.0063 (1.914)*
Final extraction (%)	65
Mean thickness (a), cm	0.007

* The datum shown in parentheses was calculated in this report

Table 15. Particle-size distribution (from Ilie et al., 1983)

Species (mm)	100	80	50	40	25	20	10	5
%	21.25	8.75	26.25	5.75	13.5	2.5	8.75	13.25

Table 16. Particle-size distribution: semi-industrial stage; G_T = 50,000 tons (from Ilie et al., 1983)

Species (mm)	300	150	70	50	15	6.5
%	13.00	28.53	19.33	10.74	25.90	2.50

Table 17. Metal extraction rates for different values of r

r, (t/(m^3/day))	25	50	75	100	125	150
V, (g/L/day)	0.0102	0.0204	0.0306	0.0408	0.0510	0.0612
v, (from Ilie et al.)	(0.0946)	(0.1292)	(0.1938)	(0.2584)	(0.3231)	(0.3877)
q(L) (m^3/dav)	2000	1000	666.66	500	400	333.33
Extraction (kg/day)	20.4	20.4	20.4	20.4	20.4	20.4

Note: Using the data of Ilie et al. (1983) and folowing step by step the corrected equations, the same values of $Q'_{M(l)}$ and $Q'_{M(L)}$ are found, but the values for V do not correspond to those given by Elie et al. (1983).

From Table 17 we can choose the quantity of solution keeping the same mean daily rate of enrichment but maintaining a more realistic solid/liquid ratio. In the literature (see references 1, 2 and 3 in Ilie et al., 1983), the flow of solution is recommended to cover the range of 6 to 10 liters per ton for 24 hours. Thus, for 400 m^3 of solution for 24 hours with a ratio r = 125 and a mean enriching rate of 0.0510 g/L/day, a flow of 8 liters per ton per day is obtained. Considering a safety factor to account for possible losses, 500 m^3 are recommended for use.

Using the basic parameters calculated, and keeping the same general conditions of the previous stage, a guideline can be presented for estimating the basic capacity and the pumping necessary and for storage of chemicals and products of leaching. Thus the semi-industrial installatioin may consist of:

A 50,000 tonnes dump of low-grade mineral.

A system to collect the pregnant solution which consists of channels feeding into collecting basins (sumps) of 500 and 5,000 m^3.

A sedimentation system composed of a net of conduits and appropriate launders to handle 500 m^3 per day.

A system to convey pregnant solution utilizing a pump with a capacity for handling 50 m^3 per hours during daily operation for 10 hours.

A launder for precipitating copper.

A platform for air- drying the copper sediment.

Extras, such as storage reservoirs, back-up systems, etc. A plant according to the scheme just described can be built by utilizing available technology for each one of the steps indicated.

For an economic analysis of microbiological leaching, it is necessry to experiment on three levels with several lots of ore. Thus a methodology is needed for scale-up from one stage to the next. To this end, equations to calculate the interrelationships between known important parameters have been worked out to characterize the process. They will allow an estimate of the scaled-up values for the desired size. In the example above, the basic theoretical equations were applied to a specific case (Ilie et al., 1983). The methodology, even though far from perfect, will allow the calculation of a first approximation and will make easier the recognition of the elements of a technological scheme for installations of larger size.

A word of caution must be added at this point. The degree of the first approximation obtained by this methodology depends to a very large extent on the validity of the assumptions made, namely that physicochemical and biological factors remain constant.

Dump and heap bioleaching are the most difficult types of processes to scale up because of the wide variations in particle-size distribution, ore homogeneity, dump or heap porosity, prevailing environmental conditions, over which the designer has very little or no control at all. However, all efforts to develop a methodology that will at least give estimates of the most important quantities involved is worthwhile because of the very low investment needed.

4.4.3 Leaching of Ore Concentrate

The methodology outlined in section 4.4.2 can be modified to take into account controlled bioleaching of ore concentrates by introducing the appropriate concentration levels (C) and/or a correction factor for homogeneity in the case of pre-ground ore concentrates, which should reflect the improvement of the controlled conditions over dump or heap leaching.

4.4.3.1 Block Diagram. Unit Operations Identification

During the last two decades, several versions of the bioleaching process have been proposed (Zimmerley et al., 1958, 1967, Duncan et al., 1967; Short and Parkinson, 1983). A generalized block diagram of sulfide-ore bioleaching is presented in Fig. 5. It includes steps of mineral ore pretreatment followed by acidification or conditioning and mixing with recycled ore residue. The mixture enters a leaching tank in which nutrient solution and/or inoculum are added, if necessary. After leaching, the ore residue is removed from the tank and separated from the pregnant liquor. A part of the residue is reground for recycling, the rest being discarded. Dead biomass should be properly disposed. The pregnant solution is passed to a recovery stage in which electrolysis is one method of recovery. Spent electrolyte is treated and recycled.

In the block diagram in Fig. 5, the following unit operations can be identified:

Crushing and grinding;

Sizing;

Flotation separation;

Mixing;

Leaching;

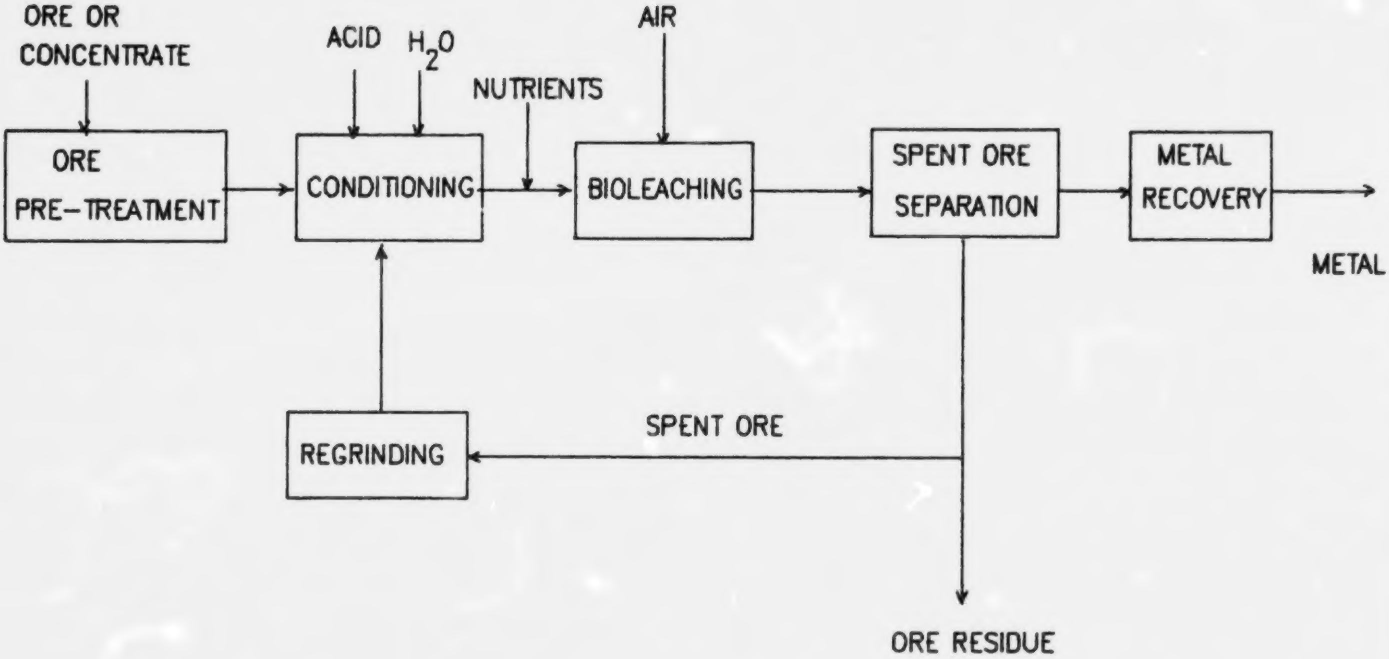
ORE OR CONCENTRATE
ORE PRE-TREATMENT
ACID
H_2O
CONDITIONING
NUTRIENTS
AIR
BIOLEACHING
SPENT ORE SEPARATION
METAL RECOVERY
METAL
SPENT ORE
REGRINDING
ORE RESIDUE

Figure 5 Block diagram of a generalized bioleaching process

Sedimentation;

Filtration;

Solvent extraction, precipitation or electrolysis.

Scale-up criteria and potential problems will be indicated for each one of these unit operations.

Crushing and Grinding

For these unit operations, the main criterion is particle size and the corresponding surface-area/volume relationship. The surface-areea/ volume ratio is particularly important for the later leaching step, whereas the particle-size distribution might prove crucial for safe and economical storage and handling. Engineering data on standard practice of ore and mineral crushing and grinding are available from several souerces such as Perry's Chemical Engineers' Handbook (Perry, 1975). Separate scale-up problems specific to the bioleaching process are expected if a particle-size distribution suitable for operation has been previously determined.

Sizing

After the crushing and grinding operations, it is often necessary to monitor the particle-size distribution of the solids before they can be bioleached. Depending on the desired particle-size distribution and the size-range allowed, sizing can vary from a very simple screening to a sophisticated cyclone treatment (Perry, 1975). Care must be exercised not to impose excessively conservative limitations on particle-size distribution because the advantages thus obtained may very well be overwhelmed by the added costs incurred in appropriate crushing, grinding, and sizing operations.

In general, the cost for cyclone separation can be twenty times more expensive than that for screening. But with the recent development in centrifuge technology, the use of a cyclone may be more attractive in the future.

Flotation

Density differences between the liquid phase and one of the solid components of a mixture can be used to separate the solid component from the mixture by froth flotation. Scale-up criteria for this process may include adequate froth levels, flotation area, and tank depth. Extra vigorous agitation and/or aeration may be required for effective scale-up of the flotation operation. This in turn may be considered an advantage because it facilitates aeration.

Flotation has been suggested as a method of separation for recycling of partially leached chalcopyrite by McElroy and Brynesteyn (1978).

Mixing

Mixing is a process that involves intermingling of two or more separate components to form a more or less uniform mixture. The degree of uniformity of the mixture is the main criterion for scale-up.

It is a very important to determine the best slurry composition for a successful leaching process. Achieving that composition might, however, prove to be difficult because in mixing, the product often consists of two or more easily identifiable phases, each of which may contain individual particles of considerable size. It must also be cvonsidered that large amounts of mechanical energy are needed for mixing.

Since bioleaching of sulfide ores by acidophilic bacteria is an aerobic process, oxygen concentration levels and oxygen transfer rates must be taken into account. This may or may not be considered as part of the mixing operation depending on equipment design and/or availability. It may require consideration of whether special aeration devices should be added or not.

Some common specific criteria for mixing used in scale-up operations are:

a. Constant impeller speed

b. Constant volumetric power input

c. Equal mixing times

d. Similar Reynolds number or momentum factors

If dissolved oxygen is one of the important factors, keeping the volumetric oxygen transfer constant should also be considered (with or without extra aeration devices).

Mathematical procedures for scale-up of mixing involving the factors above are discussed by Wang (1979).

Sedimentation

Sedimentation is probably the least expensive method available to separate solid particles from a suspension. This operation is frequently encountered in different stages of the bioleaching processes. McElroy and Brynesteyn (1978) mention feed and slurry thickeners in which sedimentation of the heavy particles concentrates (thickens) the material.

Scale-up criteria must inlcude geometric considerations such as settling area, tank depth and inclination (if any). Particle Reynolds number as well as the type and rate of settling must also be taken into account (Weber, 1972) (see Figs. 6 and 7). Determination of settling rates is crucial if the settling tank is designed for continuous operation.

Several important external influences on particle behavior, such as eddies, temperature variations, imperfect distribution of velocity vectors, etc. in a full-scale operation are neglected in most settling experiments. Safety factors should be estimated to take these influences into account (Perry, 1975; McCabe and Smith, 1976).

Filtration

Filtration for removal of solid particles from a liquid by passing the suspension through a filtering medium on which the solids are deposited, ranges from simple straining to highly complex separations. Filtration should be used in bioleaching as a means of clarifying the pregnant liquor prior to recovery of the metal and also as a reconditioning step for the recycling of the leach solution.

Standard scale-up procedures (Perry, 1975) for this operation should not harm a good design. Caution must be exercised, however, in the selection of a filter medium that is to be used, because it must be resistant to possible miocrobial and chemical attack. Any filter medium must have good solids retention; must not plug easily; be physically strong to withstand process conditions; allow easy cleaning and complete cake discharge; and have a reasonable cost.

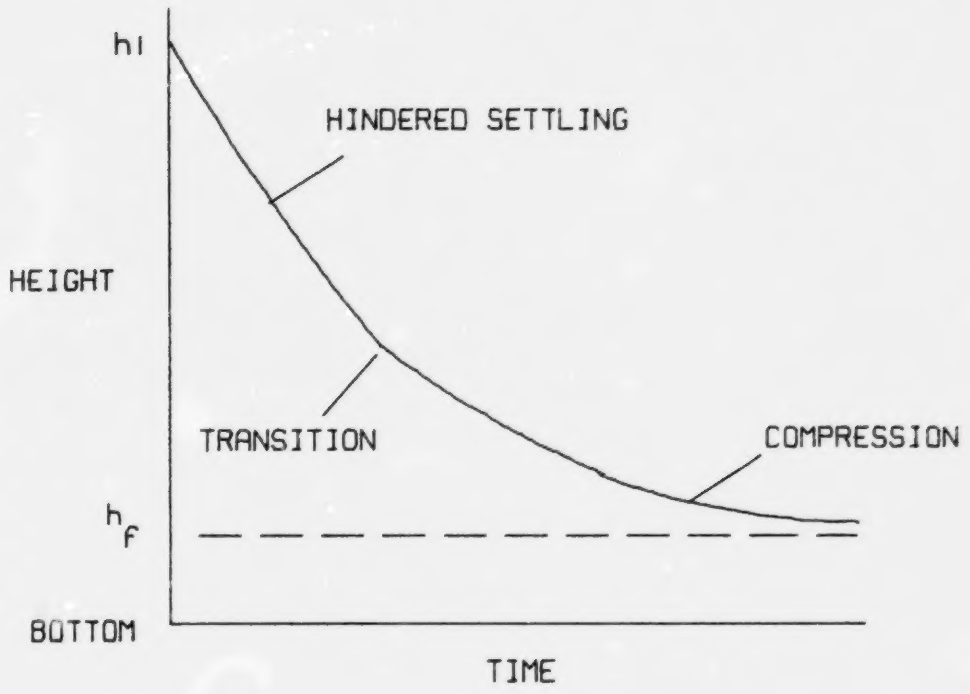

Figure 6 Height of interface in zone settling

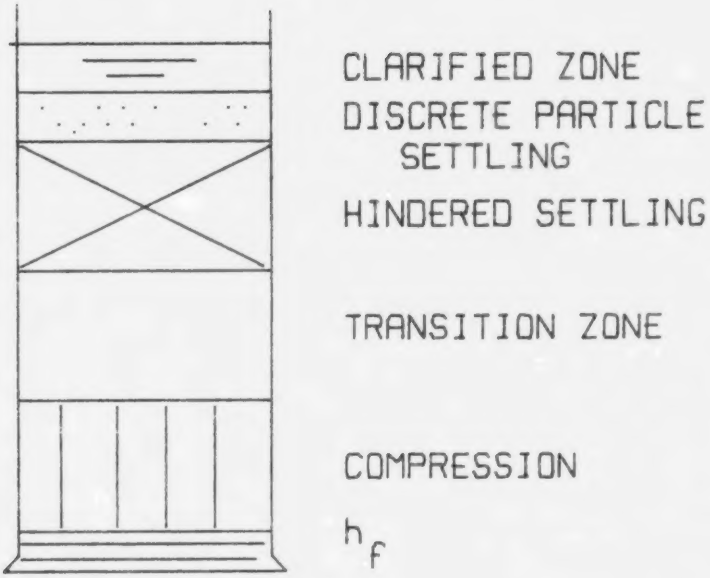
CLARIFIED ZONE
DISCRETE PARTICLE SETTLING
HINDERED SETTLING
TRANSITION ZONE
COMPRESSION
h_f

Figure 7 Zone sedimentation

Recovery

Three procedures for metal recovery from pregnant solution are posssible. They are solvent extraction, precipitation, and electrolysis.

Solvent extraction has been used successfully in the uranium industry (Hazen, 1960), and in the recovery of high purity vanadium (Swanson et al., 1961). Recent developments have also proved successful in hydrometallurgical treatment of other metal ores by the use of specific extractants (e.g., LIX64N for Cu).

A simplified outline of a liquid ion exchange unit is given in Fig. 8. There are a few technical problems in the scale-up of this operation. The concentration of metals in the solution, the value of the metal being recovered and its flow throughput, and upstream and downstream aspects of the overall process may contribute singly or in combination to a decision on the possible use of solvent extraction. Detailed discussions of these aspects are given by Ritcey and Ashbrook in Schweitzer (1979). The recovery cost of this operation can be as low as 2 cents per pound of copper for copper concentrations between 25 and 30 grams per liter (Schweitzer, 1979).

Precipitation is a method used to recover a metal like copper from pregnant solution. It involves a reaction between scrap iron and Cu^{2+} in the pregnant solution such that the Cu^{2+} precipitates as metallic copper while the iron dissolves as Fe^{2+}. The following equation describes the reaction:

$$Fe^{o} + Cu^{2+} \text{------>} Fe^{2+} + Cu^{o}.$$

The reaction is carried out in a launder. This operation was labelled cementation by Zimmerley et al. (1958). Standard procedures for scale-up are not available.

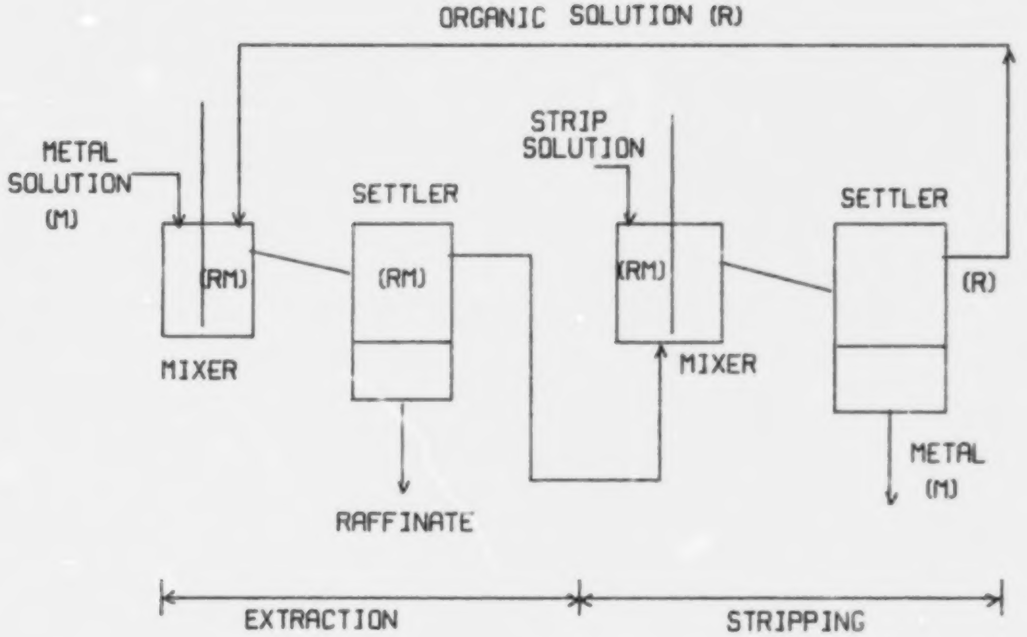
ORGANIC SOLUTION (R)
METAL SOLUTION (M)
SETTLER
STRIP SOLUTION
SETTLER
(RM)
(RM)
(RM)
(R)
MIXER
MIXER
RAFFINATE
METAL (M)
EXTRACTION
STRIPPING

Figure 8 Flow sheet of a liquid ion exchange process

Electrolysis

Although not a conventional unit operation, electrolysis is, however, one important means of separating and recovering metals from solution. The clear advantage of this process is the purity of the product obtained, but the main disadvantage is that it may be very expensive (up to 1/3 of the total cost of the leaching operation according to McElroy and Bruynesteyn (1978). Standard procedures for scale-up are not available and the potential problems are sizing and geometry of the electrolysis cells as well as the electrodes. Also, as the metal deposits on the cathode, it creates polarization problems affecting the recovery of the metal; electrode conditioning may prove crucial.

4.4.3.2 Scale-Up of Ore Concentrate Leaching

In the leaching of ore concentrate, the methodology for calculating the surficial metal concentration (equations 5 to 7 in section 4.4.2.2) may have to be modified. For example, the parameter (a) may be a function of particle size and metal concentration. Moreover, additional parameters such as the size of the leach tank, the duration of leaching, and the aeration/agitation rate have to be considered. Otherwise, the rest of the scale-up procedure (equations 8 to 14 in section 4.4.2.2) can be applied to ore concentrate leaching. However, the procedure can only be used for batch operations. In continuous/semi-continuous operations, the scale-up criteria are very different, depending on the mode of operation (continuously stirred tanks, packed beds, or spouted beds). Since these operations are still in the experimental stage, there has not been any scale-up methodology published. Nevertheless, the scale-up factor of surficial metal concentration remains critical.

5.0 MARKET ASSESSMENT AND FORECAST

5.1 METAL EXTRACTION AND RECOVERY

5.1.1 Commercially Operating Microbial Metal Processes

Microbial metal extraction has been and is being practiced on an industrial scale world-wide in the form of dump-, heap-, and/or in-place leaching of low-grade copper-sulfide and uranium ores and ore tailings by major mining companies. The leaching operations are generally run in an empirical fashion, i.e., the natural acidophilic, iron- and sulfur-oxidizing bacterial flora associated with the ores are selectively stimulated to grow and promote leaching by

(1) watering the ore, whether in dumps, heaps, or in a fractured ore body,

(2) insuring acid conditions (some sulfuric acid may have to be supplied from external sources),

(3) insuring an adequate nutrient supply, especially in respect to nitrogen (this is not usually a problem) and

(4) insuring adequate aeration of the process. No special control is exerted over the microbial leaching activity to separate it from purely chemical leaching activity, i.e., the oxidizing action of acid ferric sulfate. The leach process is generally cyclic, as explained in section 3.1.1.1. No special effort is made to insure that an optimal leaching flora develop in these leach processes. Inoculation with special strains is not practiced and in most instances would not be successful because of the likelihood that the bacteria in the inoculum could not compete with the native flora and would be outgrown by it.

Microbial leaching of uraninite-containing ores has been or is being practiced chiefly in Canada at present. As in the case of dump-, heap-, and/or in-place leaching of copper, uraninite is leached with the aid of an acidophilic, iron-oxidizing bacterial flora.

Pioneering studies were undertaken in the 1960's in the Elliot Lake region of Ontario, Canada (Fisher, 1966; Eng. Min. J., 1967). Companies involved in these studies included

Stanrock Uranium Mines, Ltd., Stanrock, Ont., Canada
(Elliot Lake Mine)

Rio Algom Mines Ltd., Toronto, Ont., Canada
(Milliken Mine; Lacnor Mine)

Dennison Mines Ltd., Toronto, Ont., Canada
(Elliot Lake properties)

From 1977 to 1983, Agnew Lake Mines, Ltd. of Toronto, Ont., Canada, recovered some uranium by bioleaching. This leaching was practiced in an empirical fashion, as in copper leaching.

In the case of copper, microbial leaching has been estimated to contribute from 10 to 20% annually to the total copper produced (Brierley, 1982; Lundgren and Malouf, 1983; Tsuchiya et al., 1974). In the case of uranium, no reliable production figures for bacterial leaching are available, but we estimate the contribution to be no greater than that for copper leaching. Most uranium is extracted from milled ore by purely chemical leaching with dilute sulfuric acid or alkaline carbonate solution.

Microbial metal extraction of low-grade ore and tailings by heap-, dump-, or in-place leaching enables a mining company to increase the total recovery of a metal value from an ore deposit beyond what would be possible by conventional methods used for high- grade ores (i.e., by grinding, flotation, pyrometallurgical extraction) at a reasonable cost.

5.1.2 Impact on Conventional Processes

Depending on the quality of the ore availble, the location of the ore body, and any environmental regulations which could raise severely the cost of pyrometallurgical extraction, biohydrometallurgical extraction could contribute from upwards of 10% to the total metal recovered. It could, in the case of extensive depletion of rich ore bodies, contribute upward of 50% to the total metal recovery.

When metal values in an ore fall below a minimal value (in the case of copper sulfide ore, below 0.4%), it becomes too expensive to separate them by crushing and milling the ore, separating the metal values by flotation, and extracting them by smelting because the metal-to-gangue ratio is too unfavorable. Too much waste would have to be handled in proportion to the amount of metal value recovered. Bioleaching under those circumstances becomes economically very attractive.

The location of an ore body very far from an ore processing plant in an unsettled, geographically remote location, or in an under-industrialized nation, may make it cheaper to recover metal values by bioleaching (heap-, dump, and/or in-place leaching). In an industrially undeveloped nation, leaching of ore concentrate, whose production would require ore crushing, milling, and beneficiation by flotation, could be more economic than smelting, especially if electricity were cheap because of extensive hydroelectric power generation. This would permit separation of the metal values from pregnant solution by electrolysis with the result that the metal so produced would be of reasonable high purity. Environmental regulations in an industrialized nation could make the modification of existent, air- polluting smelters more expensive than building a new facility for bioleaching of ore concentrate.

To be competitive with smelting, a bioleaching process of ore concentrate would have to be reasonably rapid, require inexpensive reagents in small quantities, permit recycling of reagents (especially water), be semi-continuous and, if possible, semi-automated, have low power consumption, and use as uncomplicated equipment as possible made from inexpensive but durable materials.

5.2 FORECAST OF MARKET IMPACT TO 1990

5.2.1 Metals Likely to be Extracted and Tonnages

Table 18 lists likely U.S. and world metal requirements in the years 1990 and 2000 and an estimated annual growth rate of metal requirement between the years 1978 and 2000. Only those metals are included for which technological (though not necessarily economically feasible) processes of biohydrometallurgical extration and/or bioconcentration exist. The estimates for 1990/2000 were taken from Bulletin 671, titled "Mineral Facts and Problems 1980", published by the Bureau of Mines of the US Department of the Interior. Particular note should be taken of the fact that, according to the Bureau of Mines, market demand requirements for gold, silver, and sulfur are likely to experience a shortfall by the year 2000. Recycling processes utilizing bioleaching and/or bioconcentration processes (Tables 1, 2, 7, 8, 9 and 13) may become of great importance for these elements. For example, sulfur can be generated biologically from sulfate (e.g., from acid mine drainage, seawater, gypsum) by bioreduction to hydrogen sulfide

TABLE 18. TOTAL YEARLY METAL RECOVERIES IN THE YEARS 1990 AND 2000 AND ESTIMATED ANNUAL GROWTH RATE FROM 1978 TO 2000(d)

Metal	Market(e)	Requirement 1990	Requirement 2000	Rate of Growth (annual) 1978–2000	Estimate of Quantity Produced in 1990 By Microbial Means(a)		
					Quantity	Unit Value(b)	Total Value ($ Million)
Ag	U.S.	225,000,000 tr oz	310,000,000 tr oz	3.1%	45,000,000 tr oz	$ 12 per tr oz	$ 540
	World	665,000,000 tr oz	840,000,000 tr oz	2.3%	133,000,000 tr oz	12 per tr oz	1596
Al	U.S.	11,400,000 tons	17,200,000 tons	4.4%	1,710,000 tons	1600 per ton	2736
	World	38,000,000 tons	63,700,000 tons	5.4%	5,700,000 tons	1600 per ton	9120
Au	U.S.	6,100,000 tr oz	7,585,000 tr oz	2.2%	1,525,000 tr oz	425 per tr oz	648
	World	62,400,000 tr oz	71,895,000 tr oz	1.4%	15,600,000 tr oz	425 per tr oz	6630
Bi	U.S.	2,960,000 lbs	3,520,000 lbs	1.7%	148,000 lbs	3 per lb	1
	World	11,870,000 lbs	14,050,000 lbs	1.7%	593,500 lbs	3 per lb	2
Cd	U.S.	5,600 tonnes	6,700 tonnes	1.8%	560 tonnes	26400 per tonne	15
	World	21,400 tonnes	26,100 tonnes	2.0%	2,140 tonnes	26400 per tonne	56
Cr	U.S.	890,000 tons	1,240,000 tons	3.4%	133,500 tons	800 per ton	107
	World	5,770,000 tons	7,950,000 tons	3.3%	865,500 tons	800 per ton	692
Co	U.S.	30,000,000 lbs	40,000,000 lbs	2.9%	4,500,000 lbs	12.50 per lb	56
	World	79,200,000 lbs	107,000,000 lbs	3.0%	11,865,000 lbs	12.50 per lb	148
Cu	U.S.	3,400,000 tonnes	4,600,000 tonnes	3.0%	1,700,000 tonnes	1760 per tonne	2292
	World	16,000,000 tonnes	23,600,000 tonnes	3.9%	8,000,000 tonnes	1760 per tonne	14080
Fe	U.S.	104,000,000 tons	120,000,000 tons	1.4%	10,400,000 tons	500 per ton	5200
	World	729,000,000 tons	945,000,000 tons	2.6%	72,900,000 tons	500 per ton	36450
Hg	U.S.	58,000 flasks	58,000 flasks	0.1%	11,600 flasks	275 per flask	3
	World	233,000 flasks	295,000 flasks	2.3%	46,600 flasks	275 per flask	13
		(1 flask = 76 lbs)					
Mn	U.S.	1,780,000 tons	2,000,000 tons	1.4%	267,000 tons	660 per ton	176
	World	15,000,000 tons	19,600,000 tons	2.7%	2,250,000 tons	490 per ton	1485
Mo	U.S.	110,000,000 lbs	167,000,000 lbs	4.2%	11,000,000 lbs	17 per lb	187
	World	390,000,000 lbs	610,000,000 lbs	4.5%	39,000,000 lbs	17 per lb	663
Ni	U.S.	400,000 tons	600,000 tons	4.0%	100,000 tons	6500 per ton	650
	World	1,620,000 tons	2,500,000 tons	4.3%	405,000 tons	6500 per ton	633
Pb	U.S.	1,900,000 tonnes	2,300,000 tonnes	2.2%	285,000 tonnes	440 per tonne	125
	World	7,500,000 tonnes	10,300,000 tonnes	3.5%	1,125,000 tonnes	440 per tonne	495
Pt	U.S.	1,350,000 tr oz	1,495,000 tr oz	2.3%	135,000 tr oz	475 per tr oz	64
	World	4,200,000 tr oz	5,495,000 tr oz	3.2%	420,000 tr oz	475 per tr oz	200
S	U.S.	21,600,000 tonnes	34,000,000 tonnes	4.6%	1,080,000 tonnes	125 per tonne	135
	World	90,000,000 tonnes	142,000,000 tonnes	4.6%	4,500,000 tonnes	125 per tonne	563
Sn	U.S.	59,000 tonnes	65,000 tonnes	0.9%	5,900 tonnes	13000 per tonne	77
	World	313,800 tonnes	343,000 tonnes	0.9%	31,380 tonnes	13000 per tonne	406
Ti	U.S.	804,000 tons	1,085,000 tons	3.1%	120,600 tons	30000 per ton	3618
	World	2,900,000 tons	4,212,000 tons	3.8%	435,000 tons	30000 per ton	13050
U(c)	U.S.	20,100,000 lbs	N.A.	N.A.	10,000,000 lbs	23.50 per lb	235
	World	80,000,000 lbs	N.A.	N.A.	40,000,000 lbs	23.50 per lb	940
V	U.S.	12,700 tons	18,400 tons	3.8%	635 tons	10000 per ton	6
	World	52,000 tons	75,900 tons	3.0%	2,600 tons	10000 per ton	26
W	U.S.	39,000,000 lbs	61,000,000 lbs	4.6%	1,950,000 lbs	13 per lb	25
	World	154,000,000 lbs	219,000,000 lbs	3.5%	7,700,000 lbs	13 per lb	100
Zn	U.S.	1,000,000 tonnes	1,800,000 tonnes	1.7%	200,000 tonnes	950 per tonne	190
	World	8,590,000 tonnes	10,600,000 tonnes	2.1%	4,295,000 tonnes	950 per tonne	4080
					TOTAL METAL VALUE OBTAINED BY MICROBIAL MEANS, 1990, U.S.		$ 17786
					TOTAL METAL VALUE OBTAINED BY MICROBIAL MEANS, 1990, WORLD		$ 93430

NOTES:

(a) Methodology: For each metal, the value of 1990 requirements likely to be met by microbial means was chosen between 5-50%. Metals currently won by microbial means (Cu, U) were assigned 50% and those metals least studied (Bi, V, W) or easily won by conventional means (S, Fe) were given lowest values (5%). Others were assigned values by GII depending on the amount of development already done, R&D still ahead, and whether metal has unusual strategic and monetary value which could hasten development.

(b) in 1983, from "American Metal Market", "Iron Age", "Chemical Marketing Reporter", and other publications.

(c) estimated from International Atomic Energy Figures. U_3O_8 equivalents.

(d) Data from U.S. Bureau of Mines "Mineral Facts and Problems, 1980" unless otherwise noted.

(e) World includes USA and Centrally-Planned Economies.

and subsequent bio- or chemical oxidation to sulfur (see section 4.1.g).

The methodology used in estimating the likely quantity of metals produced microbially is given in Table 18 (notes). The estimated quantity has been multiplied by current (1983) metal prices to give a deflated sales value for each metal. This forecast is contingent on sufficient R&D and scale-up work being undertaken in the future to make these processes a commercial reality, and on the absence of major technical breakthroughs in conventional extraction and concentration processes that could work against adoption of microbial processes. It should be regarded as highly speculative because of the large degree of risk involved in bringing these plans to fruition, and the conservative stance of many mining and ore processing firms to developing and utilizing this technology.

Copper and uranium are the two metals that will be won microbially in 1990 up to 50% of total production, we estimate. Microbial production for Cu and U is in the 10-20% range at the present time and worth about $350 million in the U.S. and $1750 million, worldwide. Other metals we feel will be particularly suitable for microbial treatment include Ag (20% of total production by microbial means), Au (25%), Hg (20%), Ni (25%), and Zn (20%). Total value of all the metals given in Table 18 obtained biologically could amount to nearly $18,000 million (U.S.) and $93,000 million (World) in 1990, up from $350 million (U.S.) and $1750 million (World) today.

To supplement information in Table 18, metals for ores of which a process of microbial beneficiation exists or is technologically feasible are listed in Table 19. Metals for ores of which microbial extraction processes exist or are technologically feasible are listed in Table 20. Lastly, metals for which processes exist by which they can be microbiologically concentrated from solution are listed in Table 21. In Table 21

Table 19. Metals for which a process of microbial ore beneficiation exists or may be possible

Ag	yes
Al	yes
Au	yes
Cr	maybe
Ti	maybe
W	maybe

Table 20. Metals for which a process of microbial extraction from ores exists or may be possible

Al = c
Bi = a
Cd = a
Cr = b
Co = a
Cu = a
Ga = a
Fe = a,b,d
Mn = b,d
Mo = a
Ni = a
Pb = a
S = a,b
Sn = d
Ti = a
U = a
V = a,c
Zn = a,c

Key:
a, oxidation
b, reduction
c, acid leaching
d, complexation

Table 21. Metals for which a process of microbial concentration from solution is possible

Bi = a,b
Cd = a,b
Cr = a,b,d
Co = a,b
Cu = a,b
Fe = a,b,c
Ga = a
Hg = d,e
Mn = a,b,c
Mo = a
Ni = a,b
Pb = a,b,c
Sn = e
U = a,b
Zn = a,b

Key:
a, precipitation by biogenic H_2S
b, adsorption or binding by cells or isolated cell components
c, by biooxidation
d, by bioreduction
e, by volatilization through biomethylation

particular note should be taken of the fact that some metals could be separated by biomethylation if the methylated metal is rendered volatile thereby (e.g.,$(CH_3)_2Hg$).

5.2.2 Market Size and Characteristics for Genetically Engineered Microbes

It is difficult to project the ultimate size of business for genetically engineered mcrobes in 1990 for metal extraction and concentration because this technology is too immature to permit accurate forecasting. The quantity of innocula needed will depend on the metal and source, the pore volume/surface area (for beneficiation/extraction), loss of potency of the culture, rate of poisoning, side reactions, etc. as well as any licensing and commercial agreements netotiated between the microbe developer and the metal producer. As a minimum, it would seem that 1% of the retail value of the metal could represent an "order of magnitude" value for sales of genetically-engineered microorganisms in 1990. This amounts to a market of $175 million (U.S.) and $900 million (world) for microorganisms for the metals listed in Table 18.

5.2.2.1 Heap-, Dump-, and In-Place Leaching

Genetically engineered microorganisms should exhibit one or more of the following characteristics:

1. improved leaching capacity over a wild-type strain;
2. improved resistance toward the toxicity of the metals to be leached;
3. improved competitiveness toward the wild-type microbial population in the leach process.

Item (3) is of special importance if the process is not to require a very massive introduction of the engineered strain at each leach cycle. Such massive inoculations would make the leach process much too expensive because it would require a special plant and expensive reagents (nutrients) to build up the inoculum to the size required by the process.

A special problem exists with the application of genetically engineered microorganisms to heap-, dump-, and/or in-place leaching processes. The problem is unauthorized acquisition of the engineered cultures by competitors. It could be fairly easy to isolate the strains from waste streams of a mining operation if they reached the outside of a mining property where they were employed for leaching. For protection against such an eventuality, it might be posssible to tag engineered strains with special genetic markers to identify them as coming from a specific source in cases of legal disputes. The genetic markers would have to be so designed that spontaneous mutation and selection or naturally occurring genetic recombination would not readily modify them or dilute them out, or that naturally occurring genetic process such as these could not readily introduce such markers in a wild-type strain.

The application of genetically engineered microorganisms to heap-, dump-, or in-place leaching is likely to have the smallest demand.

5.2.2.2 Bioleaching of Concentrates

Genetically engineered microorganisms for this type of process should exhibit traits (1) and (2) listed in section 5.2.2.2, and also (3) if it is possible that the engineered culture might be outgrown by wild-type organisms present in the system unless at least semi-aseptic conditions were observed which would make such overgrowth unlikely. Instituting semi-asepsis in a leaching process introduces, however, extra expense which could negate the economic advantage of using an engineered strain. A requirement for semi-asepsis should therefore be avoided.

As for engineered strains for dump-, heap-, or in-place leaching, these microorganisms should be genetically tagged to identify them in the case of unauthorized acquisition by a competitor.

For metal extraction from ore concentrates by bioleaching, specifically engineered cultures, if they can be shown to be superior to wild-type strains or spontaneous or induced mutants, can be expected to be in high demand. Ores from different sources may require differently engineered strains.

Utilization of engineered strains by ore processors may be as a test-tube size culture, which the ore processor then would have to build up to inoculum-size by cultivation before the culture could be introduced into the leach reactor. A 10% (v/v) inoculum with a population of 10^9 viable cells per ml will be desirable to minimize a lag before leaching starts. Such a build-up of inoculum requires step-wise transfer to successively larger volumes of culture medium at specified time intervals (optimally 1 to 2 days with T. ferrooxidans), which in turn requires a series of special culture vessels and thus a special plant for inoculum preparation.

As an alternative, a genetic engineering company might provide the additional service of distributing its engineered strain in process-inoculum size, eliminating the need for the ore processor to maintain a special inoculum-generating plant. Since the genetic engineering company is likely to offer its inoculum preparation to several different customers, the cost of producing process-inocula could be greatly reduced.

Since bioleaching of ore concentrate is best handled in a semi-continuous manner, one batch of process-inoculum could serve for an extended period of time, i.e., weeks or months.

The industrial use of prepared inocula from an outside supplier has precident in the brewing industry, where small breweries purchase their yeast inocula from a central supplier. Such yeasts can be used for a limited number of successive primary fermentations before they are discarded and a new batch of yeast is employed.

5.2.2.3 Bioconcentration of Metals With Genetically Engineered Microorganisms

Genetically engineered microorganisms (prokaryotic or eukaryotic) to be used for metal concentration from solution should exhibit one or more of the following traits:

1. an enhanced affinity for one or more metal ions by amplifying
   a. a capsule-polymer with this affinity,
   b. a cell-wall component with this affinity,
   c. a membrane component with this affinity,
   d. an intracellular component with this affinity, e.g., metallothionein;
2. enhanced metal tolerance;
3. an amplified metal transport system in its cell membrane;
4. reusability, i.e., concentrated metal(s) should be recoverable (elutable without destruction of the binding capacity of the cell components for the metal(s).
5. easy disposability at nominal cost once the concentrating capacity of the cells has been exhausted.

As in the case of cultures used for leaching ore concentrates (section 5.2.2.3), engineered cultures with metal accumulating capacity may be acquired by the ore processor in test-tube size or in process-inoculum size. In the former case, the processor would have to build up the culture size to the level required for metal concentration. The building up would be in a step-wise manner and most likely would require special culture equipment. In the latter case, the ore processor would acquire the culture in process-inoculum size from a supplier, who might be the genetic engineering firm or a firm specializing in inoculum preparation. This might be the preferred choice of an ore processor because of the cost saving involved in not having to build and operate an inoculum preparation plant.

5.2.2.4 Metal Accumulation by Purified Cell Components or Cell Products From Genetically Engineered Microorganisms

It may be more efficient and cheaper to use purified cell components or cell products from microorganisms in which the production of the components or products has been amplified by genetic engineering.

Among cell components, bacterial capsules or slimes, or cell wall components (e.g., peptidoglycan, or teichoic or teichuronic acids from gram-positive bacteria, or chitin from fungi transformed into chitosan by deacetylation) should be considered. Among cell products, metallothioneins (see section 3.2.2.2) and sidero- or cuprophores (see section 3.1.3.2) should be considered.

Economical separation and purification techniques should be available for any of these products. Their preparation on a massive scale would best be undertaken by an organization separate from the ore processor. The ore processor would purchase the desired product in bulk for metal concentration processes. The product should be reusable for a sufficient number of times to bring its cost down to manageable levels.

5.2.3 Market Size and Characteristics For Equipment and Chemical Sales

5.2.3.1 Equipment

No special equipment other than corrosion-resistant piping and pumps for distributing lixiviant on dumps, heaps, and broken ore bodies, and launders for recovery of metals like copper from pregnant solutions are needed. Demand for these items will be commensurate with mining activity.

Concentrate leaching will require the design and construction of reactor vessels, separators, grinding wet-equipment, electrolysis equipment, solvent-extraction equipment, etc. The demand for these will be dependent on the extent to which concentrate leaching becomes accepted practice in the industry.

5.2.3.2 Chemical Sales

For leaching processes involving chemolithotrophic bacteria, requirements for nutrient supplementation with inorganic chemicals will be limited to sulfuric acid as acidulant in cases where the microbes do not generate enough acid from metal sulfide oxidation, and to native sulfur in cases where it is needed for biogenic sulfuric acid for indirect leaching. Low amounts of ammonium sulfate and/or phosphate may be required to satisfy nitrogen and phosphorus demands by the bacteria.

For leaching processes involving heterotrophs, organic carbon and energy sources will be needed. They may be satisfied by molasses, wood hydrolysates, sulfite waste liquor, whey, sewage, algal debris, etc., depending on the process. Molasses concentrations in a medium may range from one to several percent by weight/volume. For large-scale processes, therefore, the demand for carbon and energy sources will be high. Nitrogen requirements may be satisfied by inorganic compounds (e.g., urea, ammonium sulfate) or organic nitrogen (e.g., protein hydrolysates or sewage).

Again, it is difficult to project the sales volume for nutrient chemicals and for special equipment for this market in 1990 because of the many unknowns and the infancy of this technology. As a rough approximation, it may be on par with the sales volume of engineered microbes, or $175 million (U.S.) and $900 million (world).

5.2.4 Geographical Growth Characteristics

5.2.4.1 Bioleaching

The practice of heap-, dump-, and in-place bioleaching can be expected to expand in industrialized nations with mineable ore deposits as the ore grade falls below a permissible limit for conventional metal extraction. This would also be true for developing nations which are presently or will be in the future mining rich or marginal ore deposits, as their ore grade decreases in quality below limits tolerable for conventional extraction.

The practice of concentrate bioleaching may be attractive to developing nations which are actively mining or contemplating mining of appropriate ore deposits but lack adequate conventional ore processing facilities and may be limited in eneregy resources to run such facilities. Assuming that concentrate can be leached at a sufficiently rapid rate in relatively simple equipment and without the use of significant amounts of expensive reagents, bioleaching of ore concentrate should be cost effective and attractive to such countries.

In industrialized nations, bioleaching of ore concentrates is likely to be cost effective only if an expensive by-product (e.g., gold) can be simultaneously recovered in sufficient quantity in addition to base metal. The leach process should also be reasonably fast (duration of days rather than weeks for 90%- plus extraction of metal values). High cost of energy and environmental constraints on pyrometallurgical extraction methods could make bioleaching of concentrates economically attractive.

In special cases, no economic or feasible alternative extraction process may exist except for a biohydrometallurgical one.

5.2.4.2 Bioaccumulation

Metal accumulation from concentrated solutions by intact microorganisms, cell components or cell products will become attractive in industrialized and developing nations when these "reagents" can be produced and distributed at costs competitive with those of purely chemical reagents, or, in the case of electrolytic separation, at costs competitive with those of electric power. To collect metals from concentrated solutions, bioaccumulators, whether in the form of cells, cell components, or cell products, are likely to be tried by industrialized nations before developing nations because of the financial risks involved in applying such untried methods of separation.

Bioaccumulators, whether cells, cell components, or cell products, if attractive for the separation of metals from concentrated solutions, will also be attractive for removal of metals from waste streams to prevent environmental pollution.

5.2.5 Impact on Energy Consumption, Metal Prices, Trade Patterns

Heap-, dump-, and in-place bioleaching, as long as it is practiced as an adjunct to conventional ore extraction processes, is not likely to have a profound effect on energy consumption, metal prices, or trade patterns. It will, however, extend the length of productive operation of a mine by allowing the lower grades of ore to be processed which otherwise would be treated as waste.

If heap- and or in-place leaching is applied to large, low-grade ore deposits, the cost of extracting the metal values would have to match or be less than those for a high-grade deposit processed by conventional means. Energy consumption for in-place leaching should be much less than for conventional mining and ore beneficiation (i.e., crushing, grinding, separating by froth flotation), but this might have to be weighed against a slower rate of metal recovery by bioleaching. Trade patterns could be affected insofar as metal importing countries, which lack high-grade ore deposits but possess significant numbers of low-grade ore deposits, started to exploit the latter and thereby decreased their corresponding metal imports, or if they satisfied their metal-requirements in this way and became exporters.

The economics of bioleaching of ore concentrates are the most debated at present. McElroy and Brynesteyn (1978) described a process of bioleaching of 200 tons of chalcopyrite concentrate per day in a continuous process in which the solubilized metal was recovered electrolytically. For this process they estimated a total treatment cost of 13.26 cents per pound of copper in 1973 dollars, compared to smelter charges of 8.5 to 14.5 cents. The same authors estimated lower costs for bioleaching of bornite and chalcocite because these minerals consume less oxygen per mole than chalcopyrite during leaching and yield more copper proportionately. Thus, bioleaching of concentrates is not likely to have a major impact on metal prices unless the process can be made cheaper than conventional ore processing.

Trade patterns will be affected by bioleaching of ore concentrates only insofar as a developing nation with high-grade ore deposits, which were not previously exploited, started to mine them and simultaneously processed them by a competitive bioleaching process.

6.0 PERSPECTIVES FOR NEW BUSINESS OPPORTUNITIES

6.1 GENERAL CONSIDERATIONS

6.1.1 Bioleaching

Biological extraction of ore concentrates and processing of wastes such as slags, slimes, galvanic sludges, fly ash, etc. will present new business opportunities if such processes can be shown to be economically sound and/or if processing of ore concentrates by smelting becomes unattractive because of environmental pollution problems. The University of Utah made a comparison for the US Department of Energy of eight hydrometallurgical and thirteen pyrometallurgcial extraction processes for sulfide ores and concluded that the total energy requirements are 5 to 15% lower for the pyrometallurgical processes for plants processing 100,000 tons per year. The cost of leaching becomes competitive, however, at plant sizes in the order of 25,000 tons per year (Parkinson, 1981). The report did not consider bioleaching specifically. The Utah study also points out that in-situ leaching of a fractured ore body seems to be the most energy-efficient, even if only 50% of the copper in the ore are recovered, but this has yet to be experimentally proven (Parkinson, 1981). Fig. 9 shows the anticipated trends in the cost of metal production by bioleaching and conventional methods.

Bioleaching of any ores containing precious metals such as gold, silver, and platinum, and low-grade ores containing metals such as Co, Cr, Mn, Mo, Ni, Pb, Sn, Ti, W, V, and Zn could be attractive. For the base metals this is especially true when one or more of them can only be obtained from low-grade deposits domestically, and a high demand exists for the metal, or if it has a strategic value.

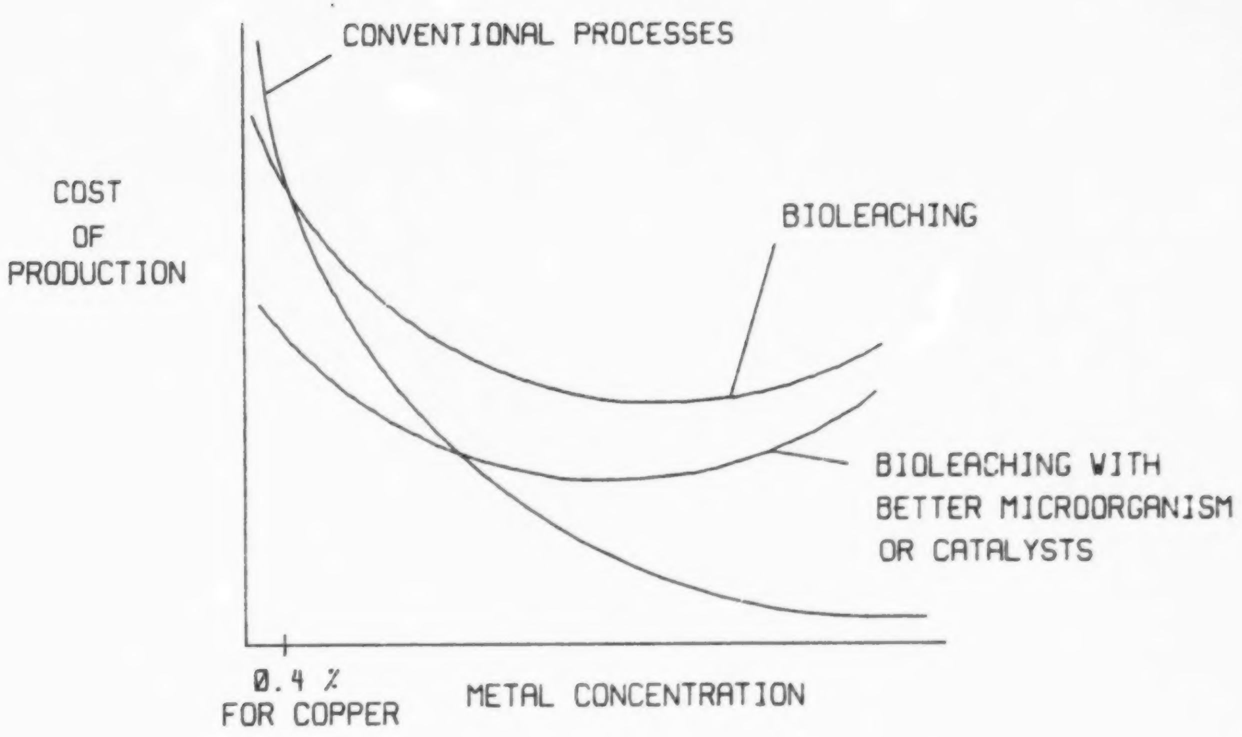

Figure 9 Cost of metal production as a function of metal concentration

Bioleaching of ores for the purpose of beneficiation can offer attractive opportunities. One example is the removal of arsenopyrites, pyrites and other sulfide minerals which interfere in the recovery of gold and silver by cyanidation (see section 4.1.e). Another is the desulfurization of pyritic coal (see section 4.1.i).

6.1.2 Bioaccumulation of Metals

6.1.2.1 Bioaccumulation From Concentrated Solution

Development of cultures, cell components, or culture products with high affinity, capacity for specific metals, and potential for reuse after metal elution offers a distinct business opportunity for use in hydrometallurgical processing. The more selective such an accumulator is, the more useful it is likely to be. Production of such accumulators must be simple and cultivation of the desired microorganisms must be on inexpensive nutrients.

6.1.2.2 Bioaccumulation From Dilute Solution

Development of cultures, cell components, or culture products with high affinity and capacity for metals for the purpose of cleaning up dilute waste streams or other polluted waters should present a good business opportunity. Such accumulators can be less selective than those mentioned in section 6.1.2.1, unless the metals recovered from dilute solution have a market value. Unless the accumulators are reused, their production must be very low in cost, and they must be easily disposable without creating a new environmental problem.

6.1.3 Genetic Engineering

Extensive opportunities exist for genetically engineering microbial strains for specific capabilities in bioextraction, biobeneficiation, and bioaccumulation of metals. Specific genetic improvements may be possible in rate of reaction by amplification of enzyme systems involved in metal extraction, in biosynthesis of bioaccumulating cell polymers, or in biosynthesis of metal complexing agents (e.g., metallothioneins, siderophores, cuprophores). Genetic improvments are also possible in respect to metal tolerance by metal extracting or metal accumulating microorganisms. In addition, genetic improvement is possible in the production of fermentation products which can act as lixiviants.

6.1.4 Bioprospecting

The possibility exists of using microorganisms as aids in the location of specific types of ore deposits (Carlisle, 1983). One way of doing this is to look in soil or on rocks for significant numbers of microorganisms with unusually high tolerance for the metal(s) for which an ore deposit is being sought. Another way is to look for specific numbers of bacteria with a specific antibiotic resistance which is known to be genetically linked with a specific metal resistance (Watterson, 1983). Although there are some limitations to this method of ore prospecting, it could reduce the cost of such a search significantly.

6.2 RESEARCH AND DEVELOPMENT NEEDS

Except for heap-, dump-, and in-place bioleaching, other processes described in this report need further R&D work before they can be fully exploited on a commercial scale. Such R&D work will consist of

1. basic research on new microorganisms or processes for bioleaching or bioaccumulation of metals. Such studies will reveal technological feasibility;

2. culture modification by genetic engineering;

3. process optimization on a laboratory scale and quantification of the effect of key parameters on process rate;

4. operation of a process on a pre- pilot- plant scale for determination of basic engineering data for further up-scaling;

5. operation of a process on a pilot-plant scale to check on efficiency of the process and on previously determined engineering constants for up-scaling; determination of the economics of the process; check on optimization;

6. development for industrial methods for preparing purified cell products or constituents, or secondary metabolites with metal bioaccumulating properties;

7. development of methods for optimal use of bioccumulators of metals;

8. economic evaluation of bioaccumulators of metals for use in metal extraction from concentrated and dilute solutions.

Several bioleaching processes for metal sulfide ores have been shown to be technically feasible. However, strain improvement by genetic engineering has not been tried in any of these instances to date to the authors' knowledge.

Real potentials exist for many other processes referred to in this study, but they need to be evaluated.

6.3 FINANCE, MANPOWER, AND TIME CONSTRAINTS FOR DEVELOPING NEW PROCESSES

Venture capital is needed for the R&D work. Full realization of economic processes is likely to require 5 to 10 years, depending on the stage in R&D at which a start is made. Process development will require the collaboration of microbiologists with a geomicrobial/biogeochemical background, chemical engineers with a microbiological/biochemical background, and mining engineers with a mineral-processing background. When the financial risk in such R&D work is considered, it would seem more attractive if such costs were shared through a consortium of interested mining and metal-refining companies. They might pool their financial resources by funding a research laboratory which would perform in-house research on specific problems as well as award contracts to university laboratories and to commercial laboratories with special expertise. All supporters would share in the research and development results generated in this way. The cost for setting up such a laboratory and the support of its activities is estimated as in the order of $10 million.

7.0 APPENDIX

7.1 REFERENCES

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7.2 GLOSSARY

Acidophile: A microorganism which grows preferentially or solely at acid pH.

Allosterism: The property of a polymeric molecule that allows it to exist in two or more configurations or conformations; in biological systems this applies particulary to certain proteins.

Autotroph: A microorganism which grows at the expense of the energy from sunlight (photosynthetic) or from the oxidation of inorganic matter (chemosynthetic), CO_2 as carbon source, and inorganic nitrogen as nitrogen source.

Bacterial cell wall: Outermost structure of a bacterial cell consisting of one or more types of polymers; responsible for maintaining cell shape; protects against external hypoosmotic stress.

Bacteriophage: A virus which attacks bacterial cells.

Barren solution: The solution in the leaching cycle after the metal value(s) have been removed.

Chitin: A homopolymer build from N-acetyl-glucosamine monomers; formed by some fungi as well as by a number of invertebrate animals.

Chitosan: A polymer derived from chitin by deacetylation.

(CH_2O): The type formula for the primary product of CO_2 fixation by autotrophs. It contains C, H, and O in the proportion found in a simple sugar like glucose ($C_6H_{12}O_6$).

Catabolite repression: Cessation of synthesis of an enzyme due to the absence of cyclic adenylic acid because of the presence of the a nutrient like the sugar glucose.

Chloroplast: An organelle in plant cells (eukaryotic) which is the site of photosynthesis.

Chromosome: A structure in a cell that carries the vital genetic information encoded into DNA. In eukaryotic cells two or more of these occur in a membranous organelle, the nucleus. In prokakryotic cells, the chromosome is free in the cytoplasm of the cell.

Conjugation: A process of transfer of genetic matter (DNA) requiring cell-to-cell contact between interacting partners.

Consortium: A mixed population of microorganisms whose physiological activity is affected by each others presence. They may be interdependent.

Constitutive enzyme: An enzyme that is always formed by a cell, regardless of whether it is needed or not.

Deoxyribonucleic acid (DNA): A heteropolymer of deoxyribose, purines, pyrimidines, and phosphoric acid; the substance which encodes genetic information and which can be transmitted in orderly fashion from one cell generation to the next.

Denitrifying bacteria: Bacteria which use nitrate in place of oxygen in their respiratory process and produce dinitrogen, nitrous oxide, or nitric oxide.

Electron transport system: A system of electron carriers and enzymes in a specific steric arrangement in a membrane which conveys reducing power from a substrate being oxidized to oxygen or some other electron acceptor; in eukaryotes located in the inner membrane of the mitochondria; in prokaryotes located in the plasma membrane.

Endospore: A bacterial spore; extremely resistant to unfavorable environmental conditions.

Enzyme: A biological catalyst, usually consisting of protein but may be combined with non-protein moieties.

Enzyme induction: Stimulation of a cell by a specific type of molecule to produce an enzyme which catalyzes the transformation of that molecule or a closely related one.

Enzyme repression: Inhibition of enzyme synthesis by a specific molecule.

Eukaryote: An organism consisting of one or more cells, each of which contain a true nucleus.

Froth flotation: A process for separating metal values from gangue by gathering the metal values in the froth of an aqueous system treated with a specific surface-active agent.

Fungus: A eukaryotic organism, frequently consisting of branched filaments, with cell walls, incapable of photosynthesis. Examples are molds and yeasts.

Gangue: The host rock of an ore which contains the valuable ore mineral(s); it is the waste produced in ore dressing.

Gene: A segment of a DNA molecule which specifies a heritable trait. Most genes code for a specific gene product (e.g., protein), but some genes have a regulatory function and do not code for a product.

Glycan: A carbohydrate polymer of six-carbon sugars.

Gram-positive: The retention of crystal violet by bacteria in the Gram staining procdure, a differential stain.

Gram-negative The lack of retention of crystal violet by bacteria in the Gram staining procedure, a differential stain.

Heterotroph: An organism which requires reduced carbon (organic carbon) as energy and carbon source.

Hydrometallurgy: A process in which metal(s) are extracted from ore in aqueous solution.

Inducible Enzyme: An enzyme which is formed by a cell only when needed, usually in response to the presence of its substrate.

Jarosite: A basic iron sulfate having the composition of $AFe_3(SO_4)_2(OH)_6$, where A may b one of a variety of cations including H^+, Na^+, K^+, NH_4^+, etc.

Launder: A tank in which metal like copper is precipitated from solution by use of sponge iron or the equivalent.

Leaching: A method of extracting metal from ore by dissolution; may include oxidation or reduction reactions.

Lysis: Breakup of a cell; disintegration of a cell.

Meiosis: Cell division among eukaryotes in which the chromosome number in the daughter cells is halved when compared to the mother cell; reduction division.

Mesophile: A microorganism which lives in a temperature range of about 20 to 43 degrees Celsius.

Messenger RNA: A type of RNA which acts as a template in protein synthesis; encoded from a specific gene on DNA.

Metal value: The metallic component of an ore.

Mineralization: A microbial process that converts organic matter into inorganic matter.

Mitochondrion: The organelle in a eukaryotic cell in which biochemically useful energy is generated by metabolic oxidations.

Mitosis: A process of eukaryotic cell division in which the daughter cells receive the same chromosome complement as possessed by the mother cells.

Mutation: A gene alteration.

Mycelium: The branched, filamentous structure of fungi and actinomycetes (a special group of bacteria).

NAD$^+$, NADP$^+$, NADH, NADPH: Abbreviations for pyridine nucleotides in oxidized and reduced form, respectively; coenzymes in some enzymatic oxidation and reduction reactions; hydrogen carriers.

Ore beneficiation: Physical, chemical or biochemical modification of an ore to increase its realtive metal content or to remove ore constituents which interfere in metal extraction.

Ore dressing: A process of preparing an ore for metal extraction.

Passivation A process by which a metal surface or crystal surface is made unreactive.

Peptidoglycan: A heteropolymer of amino sugars and amino acids found in many bacterial cell walls; gram-positive bacterial walls contain a lot of it; gram-negative bacterial cell walls contain much less of it. Some bacteria (archaebacteria) do not contain it at all.

Periplasmic space: The space between the plasma membrane and the outer membrane of a gram-negative bacterium.

Permease: A protein in cell membranes involved in the transport of specific solutes in or out of the cell.

Plasmid: An extrachromosomal genetic element composed of DNA in bacteria and some yeasts. It is 1\10 or less the size of a bacterial chromosome which has aMW $>10^9$.

Pregnant solution: The solution resulting from ore leaching which contains the extracted metal value(s).

Prokayote: A cell which lacks a true nucleus; its chromosome is not membrane bound; includes all bacteria.

Pyrometallurgy: A method of metal extraction from ore involving smelting.

Recombination: A process by which the sequence of genes on a chromosome is changed.

Ribosome: A celluar structure consisting of protein and RNA on which new protein is synthesized in a cell.

Ribonucleic acid (RNA): A heteropolymer of ribose, purines, pyrimidines, and phosphoric acid. Different types of RNA have different functions.

Secondary metabolite: A metabolic product formed after a microbial culture has gone through its growth cycle.

Siderophore: A chemical entity which complexes ferric iron.

Teichoic acid: A heteropolymer in the wall of gram-positive bacteria consisting glycerophosphate or ribitolphosphate units with alanyl and in some cases glucose side-chains.

Teichuronic acid: Like teichoic acid but including uronic acid side-chains.

Thermophile: A microorganisms which grows in a temperature range of 40 to 100 or more degrees Celsius.

Transfer RNA: A form or RNA which conveys amino acids to messemger RNA on a ribosome for incorporation into protein.

Transduction: A process of genetic modification in bacteria in which bacteriophage conveys bacterial gene(s) from a donor to a recipient.

Transformation: A process in which a DNA fragment [gene(s)] from a donor are incorporated into the chromosome of a recipient without a vector.

Wild type: A genetically unmodified organism (as opposed to a mutant).

Yeast: A fungus which exists predominantly or exclusivelly in a single-celled state.

7.3 DIRECTORY OF SOME COMMERCIAL GOVERNMENT AND UNIVERSITY LABORATORIES WITH ACTIVE INTEREST IN BIOLEACHING OF ORES, BIOCONCENTRATION OF METALS, AND/OR COAL DESULFURIZATION

7.3.1 Commercial Laboratories

7.3.1.1 U.S.A.

Advanced Mineral Technologies, Inc., Socorro, NM 87801 (Dr. Corale L. Brierley, Pres.)

General Electric Company, Corporate Research and Development, P.O. Box 8, Schenectady, NY 12301 (Dr. David S. Holmes, Molecular Biologist)

Genetics Institute, 225 Longwood Avenue, Boston, MA 02115 (R. W. Swartz, Director of Biochemical Development)

Gorham International Inc., Gorham, Maine 04038 (H. D. Olmstead, Vice President, New Business Development)

Jacobs Engineering Group, Inc., 251 South Lake Avenue, Pasadena, CA 91101-3063

7.3.1.2 Other

BC Research, Vancouver, British Columbia, Canada (A. Bruynesteyn)

Gencor Group Laboratories, West Rand, Transvaal, South Africa (E. Livsey-Goldblatt)

7.3.2 Government Laboratories

7.3.2.1 U.S.A.

Bureau of Mines, U.S. Department of Interior, 4800 Forbes Avenue, Pittsburgh, PA 15213

National Bureau of Standards, U.S. Department of Commerce, Washington, D.C. 20234 (F. Brinckman, W. Iverson, and G. Olson)

National Science Foundation, Minerals and Primary Metals Processing Program, Washington, D.C. 20550 (T. Mukherjee)

7.3.2.2 Other

Bundesanstalt fuer Geowissenschaften und Rohstoffe, 3000 Hannover 51, Federal Republic of Germany (K. Bosecker)

Canada Centre for Mineral and Energy Technology, 555 Booth Street, Ottawa, Ont., Canada K1A 0G1 (M. Silver)

Ore Research Institute, Prague, Czechoslovakia (J. Tomasek)

Warren Springs Laboratories, Stevenage, U.K. (N. W. LeRoux)

7.3.3 University Laboratories

7.3.3.1 U.S.A.

R. P. Blakemore, Department of Microbiology, Spaulding Hall. University of New Hampshire, Durham, NH 03824. (magnetotactic bacteria)

D. J. Cork, Department of Biology, Illinois Institute of Technology, Chicago, IL 60616. (bacterial conversion of sulfate to sulfur)

D. A. Crerar, Department of Geological and Geophysical Sciences, Princeton University, Princeton, NJ 08540. (acid mine drainage control)

P. R. Dugan, O. H. Tuovinen, Department of Microbiology, Ohio State University, Columbus, OH 43210. (bioleaching, bioaccumulation of metals; coal desulfurization)

H. L. Ehrlich, Department of Biology, Rensselaer Polytechnic Institute, Troy, NY 12181. (bioleaching, microbial metal concentration)

N. L. Gale, Department of Life Science, University of Missouri-Rolla, Rolla, MO 65401. (bioaccumulation of metals)

W. C. Ghiorse, Department of Microbiology, Cornell University, Ithaca, NY 14853. (iron and magnaese oxidation and reduction)

A. P. Harrison, Jr., Division of Biological Sciences, University of Missouri, Columbia, MO 65201. (acidophilic iron bacteria)

G. R. Lanza, University of Texas-Dallas, P.O. Box 688 (BE 22), Richardson, TX 75080. (bioaccumulation of metals; microbial desliming)

D. G. Lundgren, Department of Biology, Syracuse University, Syracuse, NY 13210. (bioleaching)

Betty H. Olson, Program in Social Ecology, University of California, Irvine, CA 92717. (metal toxicity; microbial genetics)

G. Stotzzky, Department of Biology, New York University, New York, NY 10003. (metal toxicity; bioaccumulation of metals)

Anne D. Summers, Department of Microbiology, University of Georgia, Athens, GA 30602. (bioleaching; microbial genetics)

K. L. Temple, Department of Microbiology, Montana State University, Bozeman, MT 59717. (bioleaching; coal microbiology)

A. E. Torma, Department of Metallurgical and Materials Engineering, New Mexico Institute of Mining and Technology, Socorro, NM 887801. (bioleaching; coal desulfurization)

D. M. Updegraff, Colorda School of Mines, Golden, CO 80401. (bioleaching)

R. F. Unz, Department of Civil Engineering, 212 Sackett Building, Pennsylvania State University, University Park, PA 16802. (coal microbiology)

J. E. Zajic, University of Texas, El Paso, TX 790968. (bioleaching)

7.3.3.2 Other

F. Acevedo, School of Biochemical Engineering, Universidad Catolica de Valparaiso, Valparaiso, Chile. (bioleaching)

A. D. Agate, MACS Institute, Pune-411 004, India. (bioleaching)

A. S. Atkins, North Staffordshire Polytechnic, Stoke-on-Tent, England. (coal microbiology)

Bose Intitute, Calcutta, India. (bioleaching)

C. Burton, Institute of Industrial Research and Standards, Dublin, Ireland. (bioleaching)

M. Zeki Dogan, Mining Engineering Department, Middle East Technical University, Ankara, Turkey.

Ebner, H., Chemietechnik, University of Dortmund, Dortmund, Federal Republic of Germany. (bioleaching)

S. N. Groudev, Higher Institute of Mining and Geology, Sofia, Bulgaria. (bioleaching)

H. Kandemir, Murdoch Univeersity, Perth, W. Australia, Australia. (bioleaching)

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